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Non-Target Analysis Using High-Resolution Mass Spectrometry to Characterize and Remediate Urban Waters

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NON-TARGET ANALYSIS USING HIGH-RESOLUTION MASS SPECTROMETRY TO CHARACTERIZE AND REMEDIATE URBAN WATERS

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Brian Ng

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To: Dean Michael R. Heithaus
College of Arts, Sciences and Education

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DEDICATION

I dedicate this dissertation to my parents, Gon Wai Ng and Jia Yu Li, my brothers and best friends Alan Ng and Phillip Ng, and the love of my life Xuerong Li, for their unconditional love, support and encouragement throughout this journey.

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ABSTRACT OF THE DISSERTATION

NON-TARGET ANALYSIS USING HIGH-RESOLUTION MASS SPECTROMETRY TO CHARACTERIZE AND REMEDIATE URBAN WATERS

by

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Florida International University, 2021

Miami, Florida

Professor Piero Gardinali, Major Professor

The first part of this dissertation will focus on the development of a simple, robust online solid phase extraction liquid chromatography high resolution mass spectrometry (SPE LC-HRMS) method followed by the use of computational software workflows for non-target analysis (NTA) of environmental samples (development). The benchmarks to assess reproducibility are not well defined for non-target analysis. Parameters to evaluate analytical performance, such as accuracy, precision and selectivity, are well defined for target analysis, but remain elusive for non-target screening analysis. In this study, quality control (QC) guidelines are proposed to assure reliable data in NTA methodologies using a simple set of standards. We have specifically evaluated method specificity, precision, accuracy and reproducibility in terms of peak area and retention time variability, true positive identification rate, intraday and interday variations and the use of QC samples to reduce false positives.

The second part of this dissertation will focus on the evaluation of different bodies of water in order to characterize different sources (application). Here we have compared electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) for the

detection and identification of organic contaminants in tap and surface waters from South Florida using a combination of Kendrick mass defect (KMD) plots and Van Krevelen diagrams (VKD). This work will lead to the creation of a unique "fingerprint" for each water body that can be used to track water quality and its point of impact. The chemical space coverage of both ESI and APCI for the purpose of non-targeted analysis was explored and documented with respect to the Environmental Protection Agency's (EPA) ToxCast chemical library. In addition, the performance of the developed NTA workflow was evaluated by analyzing 10 complex mixtures from an inter-laboratory study as part of the EPA's Non-Targeted Analysis Collaborative Trial (ENTACT).

The final part of this dissertation will focus on the development of a simple and inexpensive polydimethylsiloxane (PDMS) sponge composite for the adsorption and removal of pollutants from high flow systems. The work also explores if the polymer can be functionalized with activated charcoal to enhance its adsorption capabilities and copper to deactivate bacteria (remediation). The PDMS sponge composites worked as expected, showing adsorption potential dominated by equilibrium partitioning according to the compounds Log K_{ow}. Adding activated charcoal to the polymer improved its adsorption capabilities. Copper is a biocide and functionalizing the polymer with it deactivated *E. coli*.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL NAME
ACN	Acetonitrile
APCI	Atmospheric Pressure Chemical Ionization
APPI	Atmospheric Pressure Photoionization
CD	Compound Discoverer
dd-MS/MS	Data Dependent Tandem Mass Spectrometry
EDCs	Endocrine Disrupting Chemicals
ENTACT	EPA's Non-Targeted Analysis Collaborative Trial
EPA	Environmental Protection Agency
ESI	Electrospray Ionization
FA	Formic Acid
FESEM	Field Emission Scanning Electron Microscopy
GC	Gas Chromatography
HRMS	High Resolution Mass Spectrometry
KMD	Kendrick Mass Defect
LC	Liquid Chromatography
MeOH	Methanol
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NTA	Non-Target Analysis or Non-Targeted Analysis
PCBs	Polychlorinated Biphenyls
PDMS	Polydimethylsiloxane

QA	Quality Assurance
QC	Quality Control
RSD	Relative Standard Deviation
RT	Retention Time
S/N	Signal-to-Noise Ratio
SLR	Sea Level Rise
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
VKD	Van Krevelen Diagram
WWTPs	Wastewater Treatment Plants

CHAPTER 1 INTRODUCTION

1.1 URBAN WATERS

Urban water refers to all water which are found in the urban environment and range from a variety of sources: natural surface water and groundwater, potable water, stormwater, sewage and other 'waste' waters, waterways and estuaries in the urban landscape (DWER). Water is one of the most valuable natural resources that is essential to the sustenance of all life on earth, which we can only live for a few days without it (Falkenmark, 2020; White et al., 2010). Water quality affects all of us daily: through the water we drink from the tap, shower and swim in, and use to irrigate crops and plants with. Water quality is defined as the condition (biological, chemical, and physical characteristics) of a body of water and its suitability for use (Johnson et al., 1997; Votruba and Corman, 2020). Cities usually share one key characteristic and it is that they are full of businesses, buildings and people. Due to everyone sharing the same relative space, air and water, environmental impacts are concentrated in relatively same areas, connected by waterways (EPA; McGrane, 2016). With an evergrowing human population, there is an increasing demand for this precious resource for not only drinking, but also various economic activities such as agriculture, rearing of livestock, industrial and recreational activities (Frappart, 2013; Tyagi et al., 2013). These activities lead to large amounts of pollution entering urban waters from a variety of diverse sources, such as industrial discharge, residential/commercial wastewater, trash and polluted stormwater runoff from urban landscapes and ailing and compromised infrastructure. As urban communities often share centralized water sources, this pollution creates both environmental and public health hazards such as lower drinking water quality and bodies of water that aren't safe for recreational use (EPA; Gilliom et al., 1999).

Due to human activities that releases greenhouse gases (most importantly carbon dioxide) such as burning of fossil fuels for example, oil, gas and coal and deforestation which reduces the amount of trees available to remove the carbon dioxide produced through these actions, this has led to the growing issue of global warming. The produced gases traps infrared radiation and warms the Earth (Houghton, 2005). This warming has led to the thermal expansion of sea water and the melting of land ice (glaciers and ice sheets) which releases all the trapped freshwater (Mimura, 2013). This warming effect has led to a rise in sea level, better known as sea level rise (SLR). The resulting SLR increasing flooding incidents (storm surge and tidal flooding), seawater intrusion, ground water inundation of coastal regions (Kulp and Strauss, 2019; Rotzoll and Fletcher, 2013; Shen et al., 2019; Sweet et al., 2017) and deteriorated water quality and availability (Houghton, 2005). These flooding incidents can lead to the leakage of wastewater and anthropogenic organic contaminants of concern into storm drains and coastal water environments (McKenzie et al., 2021).

Due to the importance and increasing demand of water for both potable and non-potable use, as well as it being a finite resource, this has led to the treatment and reuse of wastewater (Ahuja, 2014; Tortajada, 2020). Emerging pollutants (chemicals not regulated and effects on both human health and environment are not unknown) have been reported in wastewater, with some chemicals such as the phthalates di-2-ethylhexyl phthalate (DEHP), benzyl butyl phthalate (BBP) and dibutyl phthalate (DBP) having regulatory status and required to be removed (Deblonde et al., 2011). However, wastewater treatment plants (WWTPs) are not effective at removing of all contaminants of concern such as 1, 2, 3-benzotriazole (BTA), a potential carcinogen, endocrine disruptors 4-benzophenone

(sunscreen agent), 4-chroloxylenol (antiseptic) and methylparaben (preservative), poly- and perfluoroalkyl substances (PFAS) and illicit drugs (Coggan et al., 2019; Kasprzyk-Hordern et al., 2008; Lenka et al., 2021; Petrie et al., 2015; Zwart et al., 2020). They may also lead to transformation products which can pose just as much threat as their parent compound, with some having been shown to be more toxic (Li et al., 2017; Schlüter-Vorberg et al., 2015; Tian et al., 2021b). In addition, WWTPs have been shown to not completely remove organic compounds such as pharmaceuticals and personal care products (PPCPs), which were found to be present in the effluent of WWTPs and could end up discharged into the environment (Batt et al., 2006; Kahle et al., 2009; Wang and Gardinali, 2013; Zhang et al., 2008). Leaking sewage infrastructure is one of the largest source of contaminants in urban waters that is not affected by WWTPs effluent discharges (Fork et al., 2021). If it is not known what pollutes our waters, it is difficult to formulate a solution for sustainable and resilient reuse. Therefore, there is an increasing need to develop analytical techniques capable of detecting the enormous number of contaminants that enter the environment which in turn, lowers water quality. The constant release of partially treated wastewaters or direct water intrusion has led to what is now called indirect potable reuse where sources of water are served primary by wastewater (Rodriguez et al., 2009).

1.2 ENVIRONMENTAL WATER MONITORING

This incomplete removal of chemicals for water reuse, have led to various agencies worldwide to establish laws to monitor or regulate such discharge. One such law is the Clean Water Act which regulates discharge of pollutants into waters bodies around the

United States. The Clean Water Act not only regulates discharge from wastewater treatment plants, but also covers discharges from other sources such as industrial facilities, agricultural activities and others (Lovett et al., 2007). In Europe, there is a similar law that aims towards protecting water bodies. The European Union's Water Framework Directive goal is to ensure no further deterioration of water bodies by setting maximum concentrations for specific water pollutants and covers 27 countries (Busch et al., 2016; Kallis and Butler, 2001). In addition to discharge from WWTPs, many other chemical compounds can enter the environment by other routes, for example, pesticides during its application, both regulated and unregulated industrial discharge into waterways, and veterinary pharmaceuticals fed to animals (Kolpin et al., 2002). As a result, this has led to the EPA regulating a list of over 65 chemical contaminants (inorganic contaminants, volatile organic contaminants and synthetic organic contaminants) and applies to all public water systems (EPA), and development of the unregulated contaminant monitoring rule which the EPA lists up to 30 unregulated contaminants every 5 years, for monitoring in public water systems (EPA). As of 2021, this list of up to 30 unregulated contaminants got updated from the previous 10 cyanotoxins, 2 metals, 8 pesticides and 1 pesticide manufacturing byproduct, 3 brominated haloacetic acids, 3 alcohols, 3 other semivolatile chemicals and 2 indicators, to 29 per- and polyfluoroalkyl substances (PFAS) and 1 metal.

Due to the constantly changing list of chemical contaminants that needs regulation and monitoring, this has led to the development of analytical techniques based on chromatographic separation and mass spectrometry for the detection and quantification of an extensive list of chemicals. Majority of the developed analytical techniques for the detection and quantification of environmental contaminants target individual or a list of

chemical compounds which can span multiple chemical classes, using specific analytical methods (Krauss et al., 2010). Typically the monitoring of environmental water bodies (rivers, lakes, streams etc.) have been done by target analysis or suspect screening for a particular contaminant of interest or screened for a list of potential contaminants of concern (Barnes et al., 2008; Baronti et al., 2000; Batt and Aga, 2005; Batt et al., 2016; Bradley et al., 2017; Busch et al., 2016; Cahill et al., 2004; Curini et al., 2000; Kolpin et al., 1998; Kolpin et al., 2002; Kolpin et al., 2004; Kolpin et al., 2006; Masoner et al., 2019; Menger et al., 2021; Pereira et al., 2016; Taylor et al., 2021; Zanella et al., 2002). These studies have found unregulated chemicals such as pharmaceuticals, personal care products, hormones, per- and polyfluoroalkyl substances, illegal drugs, new degradation products and byproducts, and other organic wastewater contaminants. Although they are capable of low concentration detection and quantification, novel contaminants and/or transformation products which may be of risk to humans and wildlife can be overlooked by these targeted methods.

1.3 NON-TARGET ANALYSIS OF ENVIRONMENTAL SAMPLES

Majority of the methods for the analysis of chemical contaminants in the environment utilizes liquid chromatography (LC) and/or gas chromatography (GC) coupled to mass spectrometry (MS). Modern advancements in mass spectrometry such as the development of tandem mass spectrometry (MS/MS), improvements from low resolution MS to high resolution (HR) MS and the coupling of HRMS/MS to LC or GC as well as chemometrics have led to the evolution of non-targeted analysis (NTA) methods to overcome this limitation (Plassmann et al., 2016). There are three main approaches for the

identification of substances using HRMS analysis: target analysis, suspect screening and non-target analysis (NTA). Target analysis requires the use of a reference standard to determine the concentration and to match the measured retention time (RT). A major drawback is that a complete target analysis cannot be done for all compounds of potential environmental concern as this would involve the purchase and measurement of hundreds or more, of chemicals for which reference standards are not always available and often requires multiple methods. Suspect screening is done when information is known in advance and does not require reference standards to indicate that a given compound may be present in the sample. This can be achieved by the calculation of the exact mass and isotopic pattern from the molecular formula of the suspected substance. Non-target analysis in contrast, assumes that no prior information is available on the compounds present in the samples and a full non-target identification from beginning to the end, starts from attaining the exact mass, isotope, adduct (if present), and fragmentation information needed (Schymanski et al., 2014b; Schymanski et al., 2015).

The NTA of environmental samples generally starts with the collection of samples to be analyzed, followed by analysis in which full scan mass spectrum (MS) data as well as tandem mass spectrum (MS/MS) fragmentation information is collected for identification purposes. After the data is acquired, one of the most important steps is the data pre-processing as it is required to make sense of the data and reduce not only the quantity, but also the complexity. This is done through a series of processes such as the detection of peaks, alignment of retention times, background subtraction using blanks. The final step in the identification of compounds of interest by NTA involves utilizing all the information obtained from the previous steps, in which the MS and MS^n data are matched

up with their respective molecular ion, isotopic pattern and fragments (Hollender et al., 2017). These NTA steps can be done by commercially available or open software or a combination of both (Fisher et al., 2021; Hollender et al., 2017; Meringer and Schymanski, 2013; Place, 2021; Tian et al., 2020; Wolf et al., 2010). Figure 1.1 below shows a generic scheme for the NTA of environmental samples.

Figure 1.1 Generic workflow for the non-target analysis of environmental samples adapted from Hollender et al., 2017.

Within the past decade, there has been an increase in application of NTA towards the monitoring of water quality worldwide (Krauss et al., 2010; Schymanski et al., 2014b; Schymanski et al., 2015; Tian et al., 2020; Tian et al., 2021a). One such example is by (Hollender et al., 2017), in which they did target analysis of 320 chemical compounds, suspect screening of 1500 chemical compounds as well as NTA of river water samples. The NTA found 2-phenyl-2-(2-piperidinyl) acetamide which is used in the manufacturing of methylphenidate, better known as Ritalin, and the synthetic byproduct tetracarbonitrile-1-propene being released into the Rhine River at large quantities from production sites upstream. This river is the drinking water source for millions of people, making it one of the most crucial rivers in central Europe. This study showed that the use of target analysis and suspect screening is not sufficient enough in the detection of contaminants of potential

concern that can be released into the environment and that NTA can supplement the limitations of the previous two techniques. But most recently, NTA has been used to solve a decades old mystery which has plagued Pacific Northwest coho salmon annually (Tian et al., 2021b). The ozonation transformation product of a tire rubber antioxidant, N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD) to 6PPD-quinone was the culprit in the deaths of spawning adult salmon exposed to stormwater, exhibiting its potential. This ozonation process occurs as intended by the manufacturers, as the antioxidant 6PPD work by reacting with atmospheric ozone found at the ground level to create a protective film to prevent the rubber elastomer from oxidizing (Tian et al., 2021b).

However, there are limitations to the use of NTA for the analysis of environmental samples. One such limitation is the extremely large number of generated features. A feature is defined as a chromatographic peak with an exact mass and its retention time (Nürenberg et al., 2019; Singh et al., 2020). Due to the large number of generated features, there is often need for data reduction strategies to help simplify the massive amount of information (Fisher et al., 2021; Knolhoff and Fisher, 2021; Peter et al., 2019; Plassmann et al., 2016; Veenaas et al., 2018). In addition, there are issues of false positive identification and false negative identification of compounds. A false positive occurs when through the NTA process, a compound is falsely identified to be present but is not actually present in the sample. Whereas a false negative is the opposite, in which a compound is not identified by the NTA process, but is actually present in the sample. The occurrence of false positives have been minimized through the use of data reduction steps, improvements to the data processing algorithms, the selected identification criteria such as mass error, isotopic pattern, signal to noise, and use of quality controls to ensure data quality (Knolhoff et al.,

2021; Myers et al., 2017; Ng et al., 2020; Vergeynst et al., 2015). Improving the data processing algorithms has decreased the occurrence of false negatives (Myers et al., 2017). It was previously reported that false negatives often occurs as a result of the peak intensity being too low (Moschet et al., 2013) as well as not having enough mass resolution (Krauss et al., 2010). False negatives can also result from sample preparation technique used (Phillips et al., 2021) as well as having criteria that is too strict for the identification process (Menger et al., 2020).

This has led to the development of a multi-phase (blinded and unblinded analysis) project by the United States (US) Environmental Protection Agency (EPA) to evaluate the NTA methods used in laboratories (approximately 30 academic, government and industry) for consistency and how well they can accurately identify unknown chemicals in samples (Sobus et al., 2018; Ulrich et al., 2019). This project is called EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) and utilizes roughly 1200 chemical compounds from the EPA's ToxCast library (Kavlock et al., 2012; Richard et al., 2016) to assemble 10 liquid mixtures, each containing roughly 100 – 400 chemical compounds each (Cite Elin's and Jon's paper) as well as a dust, wristband and serum extract, that was spiked with one of the mentioned mixtures (Sobus et al., 2018; Ulrich et al., 2019). These mixtures were given to participating laboratories and their respective NTA results reported back to the EPA to evaluate what was accurately identified, what was inaccurately identified and what compounds were not detected at all.

1.4 OBJECTIONS OF THE DISSERTATION

Objective 1: The first goal of this dissertation is to develop a NTA method to be used towards the analysis of complex environmental water samples. Currently there are no established standard quality assurance/quality control (QA/QC) criteria for NTA. Due to the lack of QA/QC and importance of data quality, one of the specific aims of this dissertation will be to introduce some quality control guidelines that can be used towards ensuring the quality of data obtained in the NTA of environmental samples. This will be done through the evaluation of the developed method in which workflow specificity, precision, accuracy, repeatability and reproducibility will be assessed. This assessment will be done using an in-house QC mixture that can be easily implemented in any analytical laboratory and customized as needed. This mixture will be strategically formulated to contain a wide range of chemical compounds that can be easily detected in both electrospray ionization (ESI) positive and negative ionization modes.

Objective 2: Once the NTA method has been developed, evaluated and the appropriate data quality assurance procedures have been established, the next aim will be the analysis of environmental water samples (surface and tap waters) collected in South Florida. This will be done using two different ionization sources, ESI and atmospheric pressure chemical ionization (APCI). This will lead to the characterization and fingerprinting of different water sources that can potentially be used towards point source tracing. In addition, the performance of the developed non-targeted analysis method will be evaluated by analyzing 10 complex mixtures as part of the inter-laboratory study by the Environmental Protection Agency's (EPA) non-targeted analysis collaborative trial (ENTACT). This study will help better understand the chemical space coverage of the use of different ionization sources

and whether APCI has complementary or superior performance than that of ESI for the purpose of NTA.

Objective 3: Lastly, the final aim of this dissertation will be the development and modification of simple and inexpensive polydimethylsiloxane (PDMS) sponges that can be made from low cost, commercially available reagents and can be applied towards the removal of organic contaminants from high flow systems by acting as a passive sampler, adsorbent and filter. These PDMS sponge composites will be porous enough to allow water to flow through them, but able to trap debris and bacteria and being developed as a sponge also gives it the added bonus of increased surface area for adsorption to occur. The PDMS sponges will be functionalized with activated charcoal to enhance its adsorption capabilities and with copper, which is a biocide, for the deactivation of bacteria. The developed sponges will be evaluated to understand their potential as a remediation tool. The effect of Log K_{ow} and their partitioning to the PDMS sponges will also be investigated, as it is expected that chemical compounds of increasing Log K_{ow} will tend to adsorb more towards the PDMS.

CHAPTER 2 ASSESSING ACCURACY, PRECISION AND SELECTIVITY USING QUALITY CONTROLS FOR NON-TARGETED ANALYSIS

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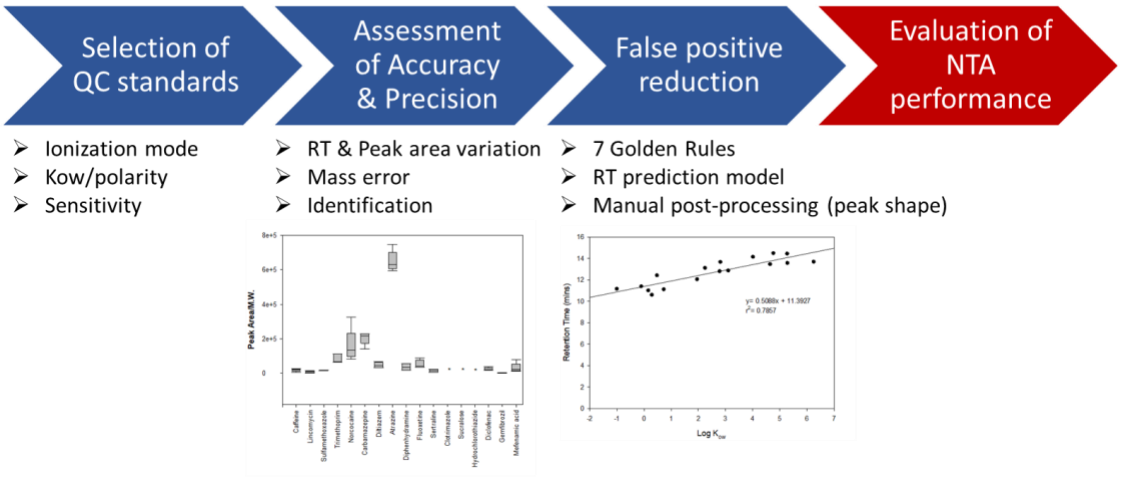

2.1 INTRODUCTION

Mass spectrometry (MS) coupled with liquid chromatography (LC) has been widely applied for the investigation of organic pollutants in environmental and biological matrices (Hernández et al., 2012; Schymanski et al., 2014b). Most approaches for the screening of environmental contaminants target individual chemical compounds or classes of chemical compounds using high sensitivity (low detection limit) quantitative analytical methods. However, novel contaminants or transformation products, which may pose a risk to humans and wildlife, are often overlooked by target methods. Recent advances in mass spectrometry as well as chemometrics have led to non-target screening approaches to help address the presence of unknown organic contaminants (Plassmann et al., 2016). Currently, three general approaches exist for the identification of substances using high resolution mass spectrometry (HRMS): target analysis, suspect screening and non-target analysis (NTA). Target analysis requires the use of a reference standard to determine the concentration and to match the observed retention time (RT). The target analysis for thousands of compounds would be unfeasible as reference standards might not always be available, especially for metabolites and transformation products. Suspect screening is done when prior information indicates that a given compound may be present in the sample. NTA, however, assumes no prior information for the compounds in the samples (Schymanski et al., 2014b; Schymanski et al., 2015).

The process of non-target screening typically entails sampling, data acquisition, data pre- and post-processing and identification (Hollender et al., 2017). The first challenge associated with NTA is the large quantity of features obtained from the high-resolution mass spectrometry acquisitions as well as prioritization of the most relevant features for

structural elucidation (Carpenter et al., 2019). Environmental samples are extremely complex and can result in a large number of peaks during data acquisition, making data processing extremely important in reducing the large quantity of data (Alygizakis et al., 2019; Hollender et al., 2017). This process has been done through peak detection, retention time alignment, background subtraction, peak intensity thresholds, mass error limits, elemental composition definition and isotopic patterns evaluation (Hollender et al., 2017).

At present, no quality assurance/quality control (QA/QC) guidelines exist for NTA performance validation. The goal of QA for analytical measurements is to generate reliable data by employing QC measures to reduce errors to acceptable limits (Taylor, 1981). QA refers to the overall measures taken by a laboratory to ensure quality of operation, while QC relates to the measures associated with the quality of individual samples or batches of samples to control the errors. A standard QC process consists of the analysis of 1) blanks, reference materials, spiked samples, blind samples, QC samples, and replicates and 2) proficiency testing (Simonet, 2005). Du et. al. applied a combination of laboratory control samples to monitor instrument performance and kept track of detector performance via mass accuracy (Du et al., 2017). Wang et. al. automatically calibrated their instrument every 5 sample injections and the sensitivity of the instrument was tested (Wang et al., 2018). Although these techniques are valid and routine instrument calibration is essential to ensure proper mass spectrometer performance, assessment of reproducibility remains a critical gap in non-targeted analysis (Hites and Jobst, 2018). The main objective of this study was to introduce simple preliminary quality control guidelines for non-target screening methodologies. Workflow specificity, precision, accuracy, repeatability and reproducibility were assessed using an in-house QC mixture that could be easily

implemented in a typical analytical lab and customized containing a wide range of compounds that can be detected in both electrospray ionization (ESI) positive as well as ESI negative.

2.2 MATERIALS AND METHODS

2.2.1 Chemicals and reagents

For NTA, the purity of all chemicals is important. All standards and reagents were purchased from commercial vendors. Water, Acetonitrile (ACN), Methanol (MeOH), and formic acid (FA) were all Optima LC/MS grade purchased from Fisher Scientific (Fair Lawn, NJ, USA). The following standards were used in this study: caffeine (>98.5% purity, Sigma-Aldrich), lincomycin (>90%, Sigma), sulfamethoxazole (>99%, Sigma), trimethoprim (>98%, Sigma), norcocaine (>99%, Cerilliant), carbamazepine (>99%, Sigma-Aldrich), (+)-cis-diltiazem hydrochloride (>99%, Sigma), atrazine, diphenhydramine hydrochloride (>98%, Sigma), fluoxetine hydrochloride (100%, Sigma), sertraline hydrochloride (>99%, Sigma), clotrimazole (>98%, Sigma), sucralose (99%, AK Scientific), hydrochlorothiazide (98.4%, MP Biomedicals), diclofenac sodium salt (100.38%, MP Biomedicals), gemfibrozil (>99%, Sigma), mefenamic acid (>98%, Sigma), atrazine D5 (97%, Dr. Ehrenstorfer GmbH) and sucralose D6 (98.5%, Toronto Research Chemicals). The QC compounds were selected to cover a wide range of polarity and K_{ow} that can be detected either by ESI in positive or negative mode. Ionization introduces bias. These were then combined to create an in-house QC mixture prepared in LCMS grade methanol at the concentration of 200 ng/mL. The chemicals and their respective molecular

formula, monoisotopic mass, octanol/water partition coefficient (log K_{ow}) and the monitored ions in ESI are listed in Table 2.1.

Table 2.1 List of quality control compounds and their respective log Kow, molecular formula, monoisotopic mass and monitored ions.

Compound	**Log K_{ow}**	**Molecular formula**	**Monoisotopic mass**	**Monitored ions**	**Retention time (min)**
Sucralose	-1.00	$C_{12}H_{19}Cl_3O_8$	396.0146	395.0073[b]	11.16
Hydrochlorothiazide	-0.10	$C_7H_8ClN_3O_4S_2$	296.9645	295.9572[b]	11.39
Caffeine	0.16	$C_8H_{10}N_4O_2$	194.0804	195.0877[a]	11.01
Lincomycin	0.29	$C_{18}H_{34}N_2O_6S$	406.2137	407.2210[a]	10.60
Sulfamethoxazole	0.48	$C_{10}H_{11}N_3O_3S$	253.0521	254.0594[a]	12.42
Trimethoprim	0.73	$C_{14}H_{18}N_4O_3$	290.1379	291.1452[a]	11.11
Norcocaine	1.96	$C_{16}H_{19}NO_4$	289.1314	290.1387[a]	12.05
Carbamazepine	2.25	$C_{15}H_{12}N_2O$	236.0950	237.1022[a]	13.11
Diltiazem	2.79	$C_{22}H_{26}N_2O_4S$	414.1613	415.1686[a]	12.80
Atrazine	2.82	$C_8H_{14}ClN_5$	215.0938	216.1010[a]	13.66
Diphenhydramine	3.11	$C_{17}H_{21}NO$	255.1623	256.1696[a]	12.86
Diclofenac	4.02	$C_{14}H_{11}Cl_2NO_2$	295.0167	294.0094[b]	14.14
Fluoxetine	4.65	$C_{17}H_{18}F_3NO$	309.1341	310.1413[a]	13.46
Gemfibrozil	4.77	$C_{15}H_{22}O_3$	250.1569	249.1496[b]	14.48
Mefenamic acid	5.28	$C_{15}H_{15}NO_2$	241.1103	240.1030[b]	14.44
Sertraline	5.29	$C_{17}H_{17}Cl_2N$	305.0738	306.0811[a]	13.57
Clotrimazole	6.26	$C_{22}H_{17}ClN_2$	344.1080	345.1153[a]	13.68

[a]Ions were monitored in ESI positive (70.6%), [b]Ions were monitored in ESI negative (29.4%).

2.2.2 Quality Control Sample Preparation

Stock solutions for each standard were prepared in MeOH and stored at -20˚C: 276.4 µg/mL caffeine, 410.4 µg/mL lincomycin, 508.7 µg/mL sulfamethoxazole, 342.2 µg/mL trimethoprim, 100.0 µg/mL norcocaine, 360.8 µg/mL carbamazepine, 388.8 µg/mL diltiazem, 1007.1 µg/mL atrazine, 394.4 µg/mL diphenhydramine, 56.2 µg/mL fluoxetine, 253.2 µg/mL sertraline, 293.7 µg/mL clotrimazole, 396.0 µg/mL sucralose, 520.0 µg/mL hydrochlorothiazide, 300.0 µg/mL diclofenac, 310.0 µg/mL gemfibrozil, 290.0 µg/mL mefenamic acid, 100.0 µg/mL atrazine D5, 1000.0 µg/mL sucralose D6. A mixture (QC+) containing: caffeine, lincomycin, sulfamethoxazole, trimethoprim, norcocaine, carbamazepine, diltiazem, atrazine, diphenhydramine, fluoxetine, sertraline and clotrimazole was made from the prepared stock solutions to contain a working solution concentration of 200 ng/mL of each standard. Another mixture (QC-) containing: sucralose, hydrochlorothiazide, diclofenac, gemfibrozil and mefenamic acid was made from the prepared stock solutions to contain a working solution concentration of 200 ng/mL of each standard. For the analysis, both QC+ and QC- mixture were diluted with LC-MS water to a final concentration of 381 pg/mL. Atrazine D5 and sucralose D6 used as internal standards were added to the QC+ and QC- respectively at a final concentration of 200 pg/mL. Blank samples, consisting of LC-MS water and labeled standards, were run daily together with the QC samples to check for background contamination.

2.2.3 High Resolution Mass Spectrometry Analysis

Before every analysis, instrument calibration was performed using the Pierce LTQ ESI positive ion calibration solution (Thermo Scientific, USA) for positive mode and the Pierce LTQ ESI negative ion calibration solution (Thermo Scientific, USA) for negative mode. Mass accuracy is checked to be less than 5 parts per million (ppm) but is routinely below 2 ppm. Analysis was carried out using a Q Exactive Orbitrap (Thermo Scientific, USA) by online-solid phase extraction (SPE) using a Hypersil GOLD aQ (20 x 2.1 mm, 12 µm, Thermo Scientific, USA). The analytical column used was a Hypersil GOLD aQ C18 polar endcapped (100 x 2.1, 1.9µm, Thermo Scientific, USA). A heated electrospray ionization (HESI) source (Thermo Scientific USA) was used, and conditions for both positive and negative mode were a spray voltage of 5.00 kV, a capillary temperature of 350˚C, auxiliary gas heater temperature of 250 ˚C, a sheath gas flow rate of 30 arbitrary units and auxiliary gas flow rate of 2 arbitrary units. Blank (LCMS water) and QC samples were analyzed in full scan positive mode with a scan range from 100.0 to 800.0 m/z at a resolution of 140,000, followed by data dependent MS/MS (dd-MS/MS) with a normalized collision energy of 30 and at a resolution of 35,000. This process was repeated using negative mode. The online SPE procedure has been described in detail before as shown in Table 2.2 and Table 2.3 below (Batchu et al., 2013).

2.2.4 Online SPE procedure

In summary, 10 mL of pre-filtered samples with the addition of internal standards atrazine D5 and sucralose D6 at concentration 200 pg/mL each were loaded in the SPE column with 98% water (A):2% acetonitrile (C) in 4.20 minutes at a flow rate of 2500

µL/min. The rotary valve switched at 4.20 minutes connecting the SPE column with the analytical column. The solvent flow through the SPE column was reversed, and the analytes were then back-flushed into the analytical column and eluted using a gradient of acetonitrile and formic acid (pH 3). The loading and elution gradient for the online SPE procedure and analytical column are summarized below in Table 2.2 and Table 2.3, respectively. At 12.20 minutes the rotary valves switched back to the initial conditions to prepare both the online SPE and analytical column for the next sample run.

Table 2.2 Gradient of pump 2 for the online SPE pre-concentration step.

Pump 2 – Online SPE						
	Time	A%	B%	C%	D%	µL/min
0	0.00	98.0	0.0	2.0	0.0	200.0
1	0.10	98.0	0.0	2.0	0.0	2500.0
2	4.20	98.0	0.0	2.0	0.0	2500.0
3	4.50	0.0	0.0	100.0	0.0	1000.0
4	6.50	0.0	0.0	100.0	0.0	1000.0
5	7.00	10.0	90.0	0.0	0.0	1000.0
6	8.00	10.0	90.0	0.0	0.0	1000.0
7	8.90	98.0	0.0	2.0	0.0	1000.0
8	15.00	98.0	0.0	2.0	0.0	1000.0

A: water, B: methanol, C: acetonitrile, D: 0.1% formic acid

Table 2.3 Gradient of pump 1 for the elution and separation step by the analytical column.

Pump 1 – Analytical column						
	Time	A%	B%	C%	D%	µL/min
0	0.00	0.0	0.0	2.0	98.0	250.0
1	4.20	0.0	0.0	2.0	98.0	250.0
2	10.00	0.0	0.0	60.0	40.0	250.0
3	12.00	0.0	0.0	100.0	0.0	250.0
4	15.00	0.0	0.0	100.0	0.0	250.0

A: water, B: methanol, C: acetonitrile, D: 0.1% formic acid

2.2.5 Data post-processing

Post-processing of the raw data files was done using the small molecule structure identification software, Compound Discoverer (CD) 3.0 (Thermo Scientific, USA) which is a commercially available and off-the-shelf software. The non-targeted workflow utilizes an extensive list of processing tools which simplifies the process of peak picking, blank subtraction, merging and grouping of features, molecular formula generation, isotopic pattern comparison, evaluation of adducts, the assignment and comparison of fragmentation pattern, and the searching of databases all in a single software rather than multiple. The information on the algorithm used for the identification is proprietary and not publicly available. Chemspider, EPA Toxcast and DSSTOX, MzCloud, MzVault, DrugBank, EAWAG Biocatalysis/Biodegradation, ACToR: (Aggregated Computational Toxicology Resource) and FDA (Food and Drug Administration) UNII – NLM databases were used. Also, the mass lists included in CD were used: EFS HRAM Compound

Database, Endogenous Metabolites database 4400 compounds and Extractables and Leachables HRAM Compound Database.

The blank was used for background subtraction, which is performed by the software when features are also identified in the blanks. The peak picking step was done with a mass tolerance of 5 ppm, intensity tolerance of 30%, S/N threshold of 3, and a minimum peak intensity of 300,000. The merging and grouping of features were done at a mass tolerance of 5 ppm and RT tolerance of 0.1 minute. The prediction of elemental composition was done at a mass tolerance of 5 ppm, maximum element counts of C90 H190 Br3 Cl6 N10 O18 P3 S5, with a maximum ring double bond equivalents (RDBE) of 40, maximum H/C of 3.5. Pattern matching was done with an intensity tolerance of 30%, intensity threshold of 0.1%, S/N threshold of 3, minimum spectral fit 30%, and a minimum pattern coverage of 80%. Fragment matching was done with a mass tolerance of 5 ppm and S/N of 3. The term “feature" is defined as the compound resulting from the data post-processing procedure that contains three elements: an exact mass-to-charge ratio of the ion formed at a certain RT and intensity of the detected ion (Pastore et al., 2018). At the end of the data post-processing step performed by the software, a list of features was obtained and additional manual data processing was performed; where the peak area of the sample must be three times higher than that of the blank, the retention time of the tentative candidate must be within 0.5 minutes based on the developed RT vs log K_{ow} model and the chromatographic peaks were checked to avoid noise integrations. Features were manually eliminated if they did not meet the criteria. After all the data processing was done, accuracy, precision, repeatability and reproducibility were assessed.

2.3 RESULTS AND DISCUSSION

In the present study we focus on the findings based on our in-house QC mixtures. Accuracy was defined in terms of true positive identification rate, i.e. the ability of CD to correctly identify the compounds present in the QC mixtures. Precision was evaluated regarding variations in retention time (RT) and respective peak area of the correctly identified compounds and expressed as relative standard deviations (RSDs). Repeatability was assessed based on intraday variations and reproducibility on interday variations. Five replicates were performed in the same day to constitute the intraday (n=5) variations and a total of fifteen replicates were analyzed over three (3) consecutive days to evaluate interday variability (n=15). Selectivity was assessed though the ability of the proposed retention time mode (RT) model based on log K_{ow} constructed from the QC samples to reduce false positives. The ability to identify the compounds and their respective RT and peak areas was based on the identification by our NTA workflow which utilizes templates provided within the software Compound Discoverer (CD). Most of this was done unattended.

2.3.1 Assessment of Accuracy and Precision

The correct identification of the compounds in the QC mixture by the available databases was evaluated. In this study, we observed that the intraday accuracy of the NTA workflow was greater than 75% identification rate for majority of the QC compounds except for trimethoprim and diphenhydramine, which were identified 60% and 40% respectively; and 3 compounds that were not identified or correctly identified (clotrimazole, sucralose and hydrochlorothiazide). Intraday accuracy results are shown in Figure 2.1A.

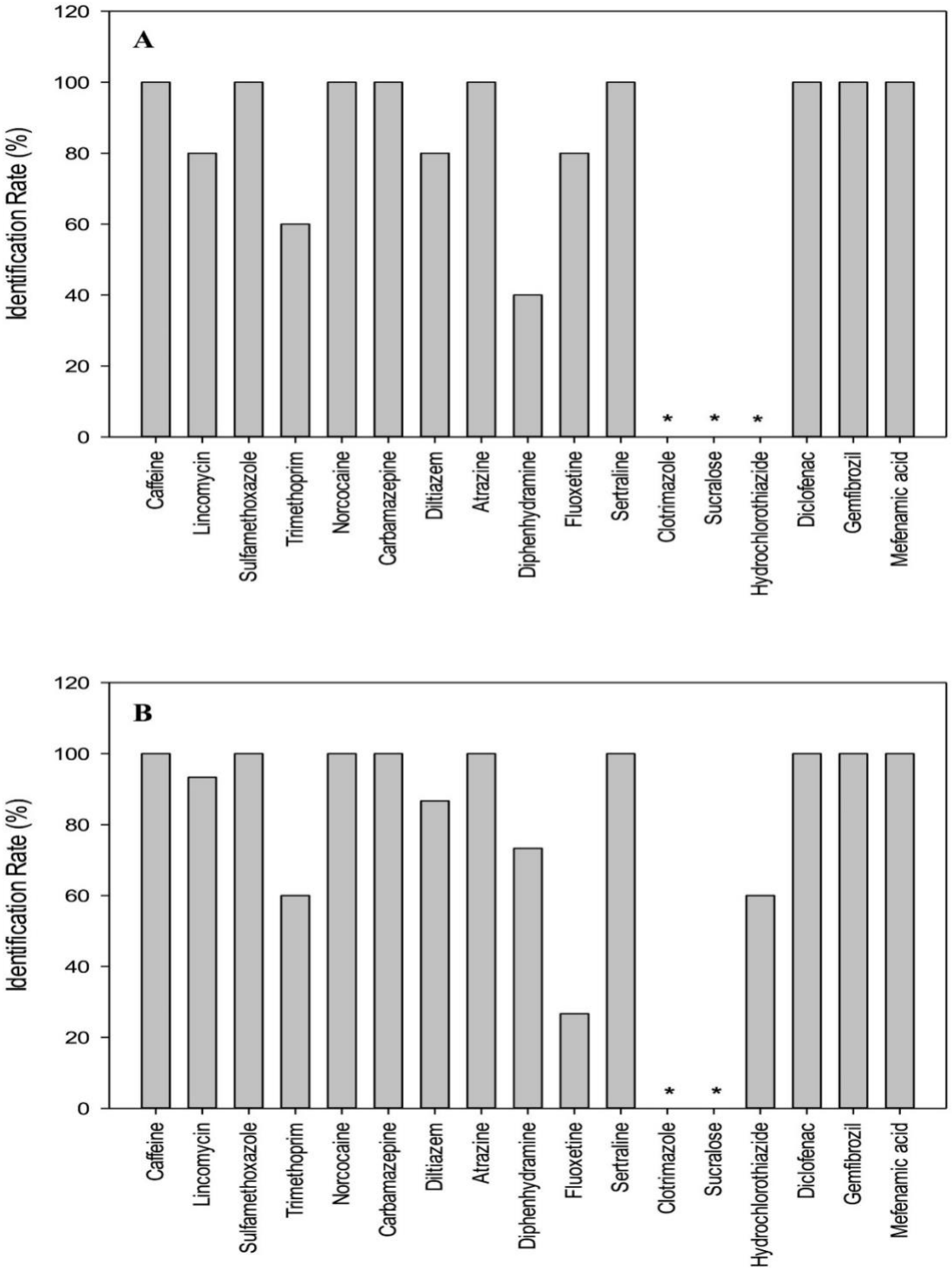

Figure 2.1 Accuracy of compound identification in the QC mixture within the same day (n=5) and over 3 consecutive days (n=15). A) intraday (top) and B) interday (bottom). (*) were not detected.

The interday accuracy of the NTA workflow was consistent with that of the intraday study, in which the majority of the identified QC compounds had an identification rate greater than 75%. Trimethoprim had a 60% identification rate throughout the course of the study, but diphenhydramine identification rate went from 40% to 73.3%, an improvement of 33.3%. The major difference was observed in fluoxetine and hydrochlorothiazide. Fluoxetine had an identification rate greater than 75%, but in consequent analyses was not identified. Hydrochlorothiazide was initially not identified, however in consequent analyses, it was identified in 93.3% of the replicates, resulting in an increase of the overall detection rate to 60%. For the entire course of the study, both clotrimazole and sucralose were not identified. The interday accuracy results are shown in Figure 2.1B. Overall, the accuracy of our NTA workflow to identify our QC mixtures was 70% or greater for most compounds except for three compounds (trimethoprim, fluoxetine, and hydrochlorothiazide), which were not always identified by the CD software, and two compounds (clotrimazole and sucralose), which were not identified at all by CD. Few compounds not correctly identified during the data post-processing workflow suggests some limitations of the software employed, which seems to be related to the complexity of the mixtures and the simultaneous search of a multitude of available databases. Compounds not identified by the software CD were checked manually via the data processing software Xcalibur and did not show any issues related to the chromatography (e.g peak shapes) and mass errors were within the established 5 ppm range.

However, taking into consideration the large number of databases searched containing hundred thousand of compounds, which included all the compounds present in the QC mixture in at least one of the databases, the number of compounds correctly

identified by the software is very promising. The inclusion of QC compounds, which are not isotope labeled standards, would potentially help us improve the reliability of the results when non-targeted analysis is being performed.

Intraday precision in terms of peak area for the identified compounds varied by compound, ranging from a RSD of 8.2% for sulfamethoxazole to 106.5% for gemfibrozil. Some were better than others, with three compounds, sulfamethoxazole, atrazine and carbamazepine exhibiting a RSD less than 30%, four compounds, diphenhydramine, lincomycin, gemfibrozil and mefenamic acid showing a RSD greater than 70% and the other compounds having a RSD between 30 to 50%. The intraday precision results for peak area are shown in Figure 2.2A. This variation in precision between compounds were also observed by Dubbelman *et. al*. in their application of their quan/qual method to a non-targeted study in which they evaluated the precision of 19 drugs across 27 samples and found that the precision varied between 12% for tolbutamide and 104% for bedaquiline (Dubbelman et al., 2018).

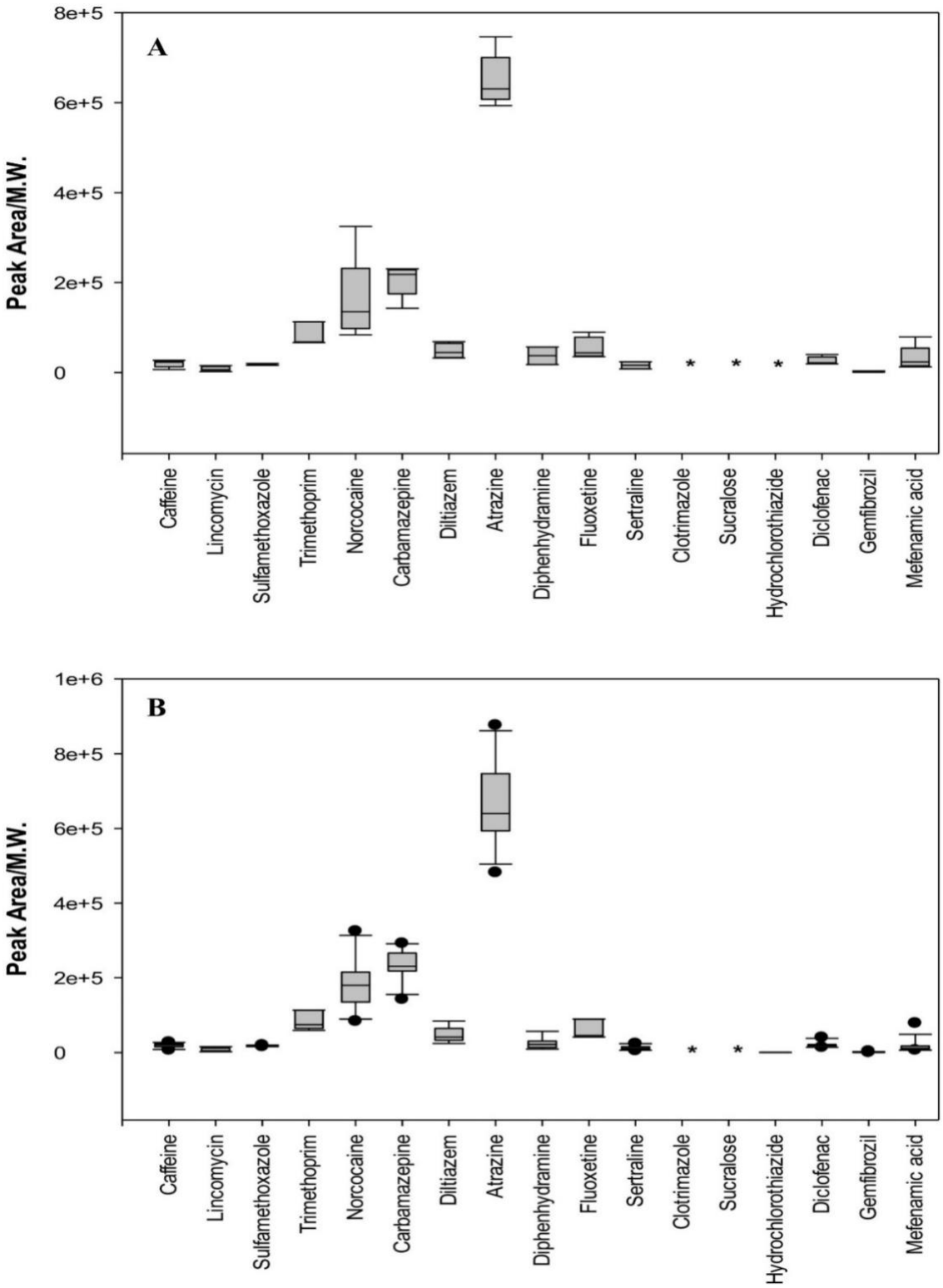

Figure 2.2 Variation in molar peak area of the identified QC compounds within the same day (n=5) and over 3 consecutive days (n=15). A) intraday (top) and B) interday (bottom). (*) were not detected.

Interday precision in terms of peak area for the identified compounds were consistent with that of the intraday. The same three compounds (sulfamethoxazole, atrazine and carbamazepine) and caffeine had a RSD below 30%. Caffeine had an improvement of 11.9% (39.5% to 27.6%). The same compounds, lincomycin, gemfibrozil and mefenamic acid had a RSD greater than 70%, with the exception of diphenhydramine which showed a RSD of 54.9%, an improvement of 18.9% (73.8% to 54.9%). Most of the compounds had a RSD between 30 to 50%. The interday precision results for peak area are shown in Figure 2.2B. RSD variation is dependent on the analyte and is largely affected by the ionization efficiency and integration algorithm by the software.

Intraday and interday precision in terms of RT for all the identified compounds were $\leq$ 5%, showing a very good reproducibility and repeatability in terms of retention time. The results for intraday precision and interday precision for RT are shown in Figure 2.3A and 2.3B respectively. A chromatogram for one of the quality control analysis is shown in Figure 2.4 below.

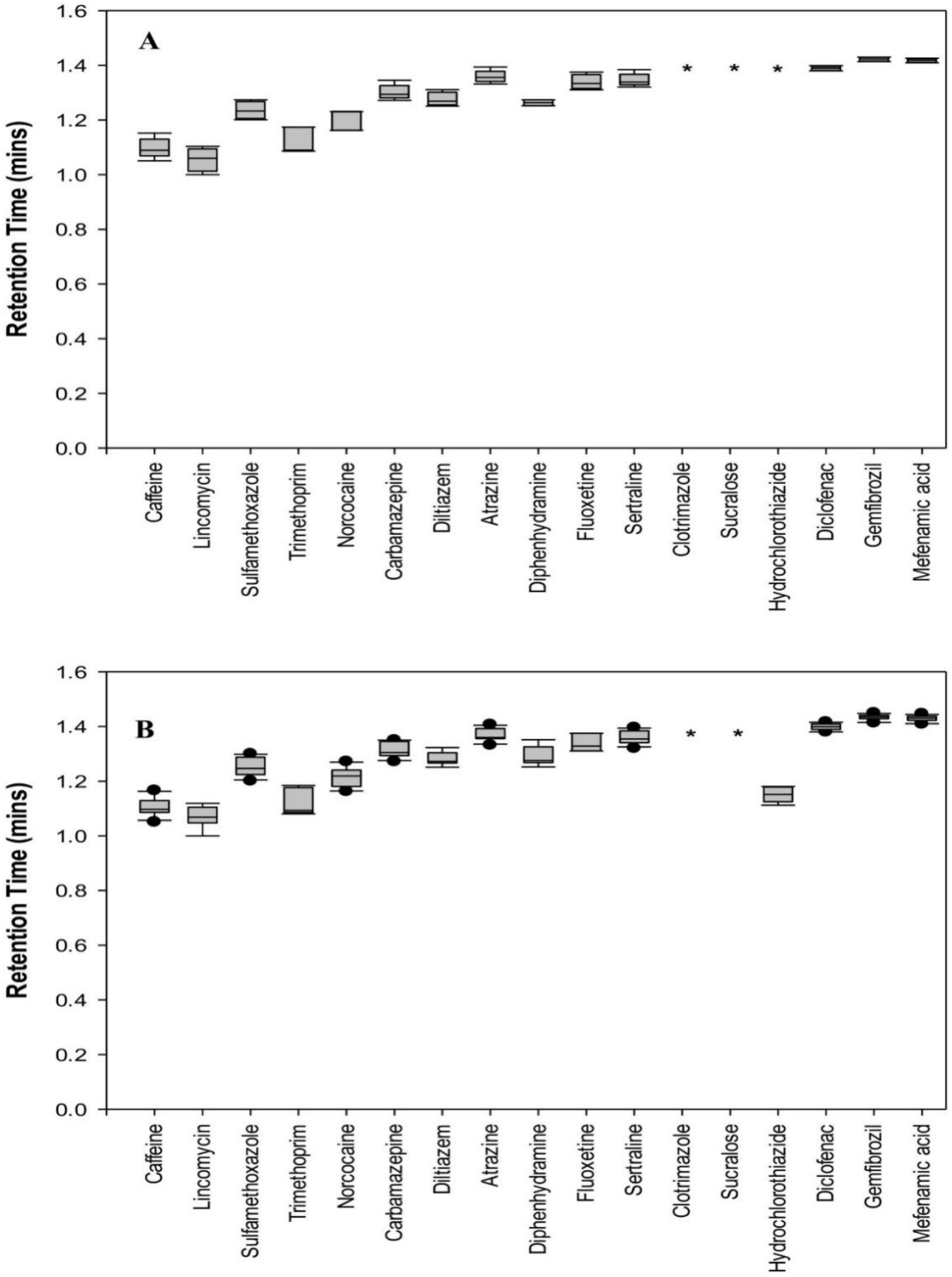

Figure 2.3 Variation in relative retention time of the identified QC compounds within the same day (n=5) and over 3 consecutive days (n=15). A) intraday (top) and B) interday (bottom). (*) were not detected.

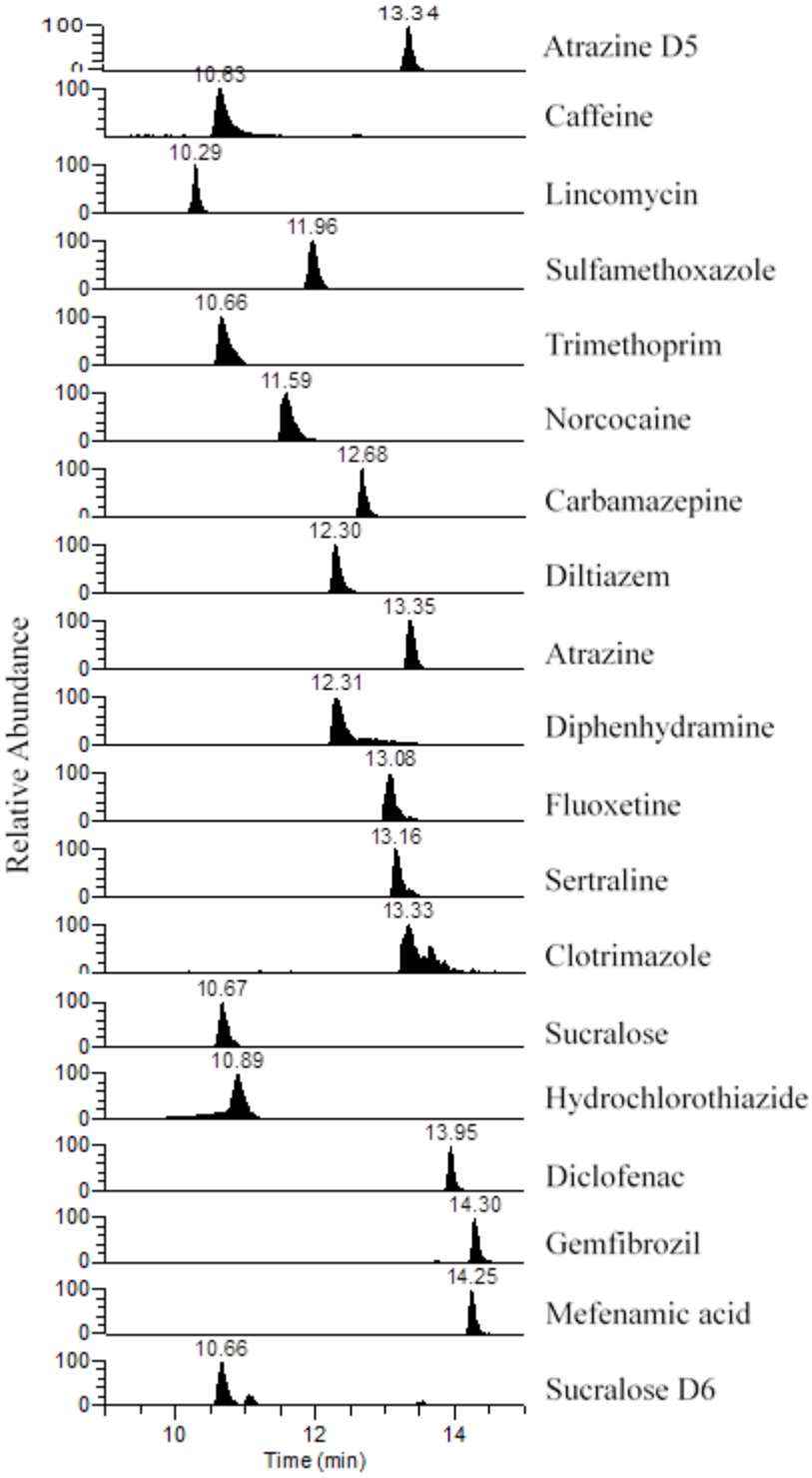

Figure 2.4 A chromatogram of a positive and negative quality control analysis.

2.3.2 Data Normalization

Data normalization approaches is very common in metabolomics to minimize technical variability attributable to batch and run order in large-scale studies (Reisetter et al., 2017). In this study, in an attempt to account for variations in instrument response and ionization and to evaluate if the repeatability and reproducibility would improve, peak area was normalized by the internal standards atrazine D5 for all compounds identified in positive mode (QC+) and by sucralose D6 for the compounds identified in negative mode (QC-). The internal standard atrazine D5 was identified in every analytical run throughout the course of the study, except for one, hence instead of n=5 for the normalized intraday peak area precision, n=4 was used and this also resulted in the normalized interday peak area precision going from n=15 to n=14. The internal standard sucralose D6 was not correctly identified throughout the entire course of the study by CD even though it was present in the database used. Therefore, the compounds analyzed in negative mode (sucralose, hydrochlorothiazide, diclofenac, gemfibrozil and mefenamic acid) were not normalized. The normalized peak area in intraday precision studies, showed that two of the three compounds (sulfamethoxazole and atrazine) still had a RSD $\leq$ 30%, with carbamazepine now having a RSD > 30% (36.1%). However, fluoxetine improved by 19.2% to a RSD $\leq$ 30%. Lincomycin retained a RSD $\geq$ 75%. Most of the compounds had a RSD between 30 to 50% and normalizing did not improve the outcome. The intraday precision results for normalized peak area are shown in Figure 2.5A.

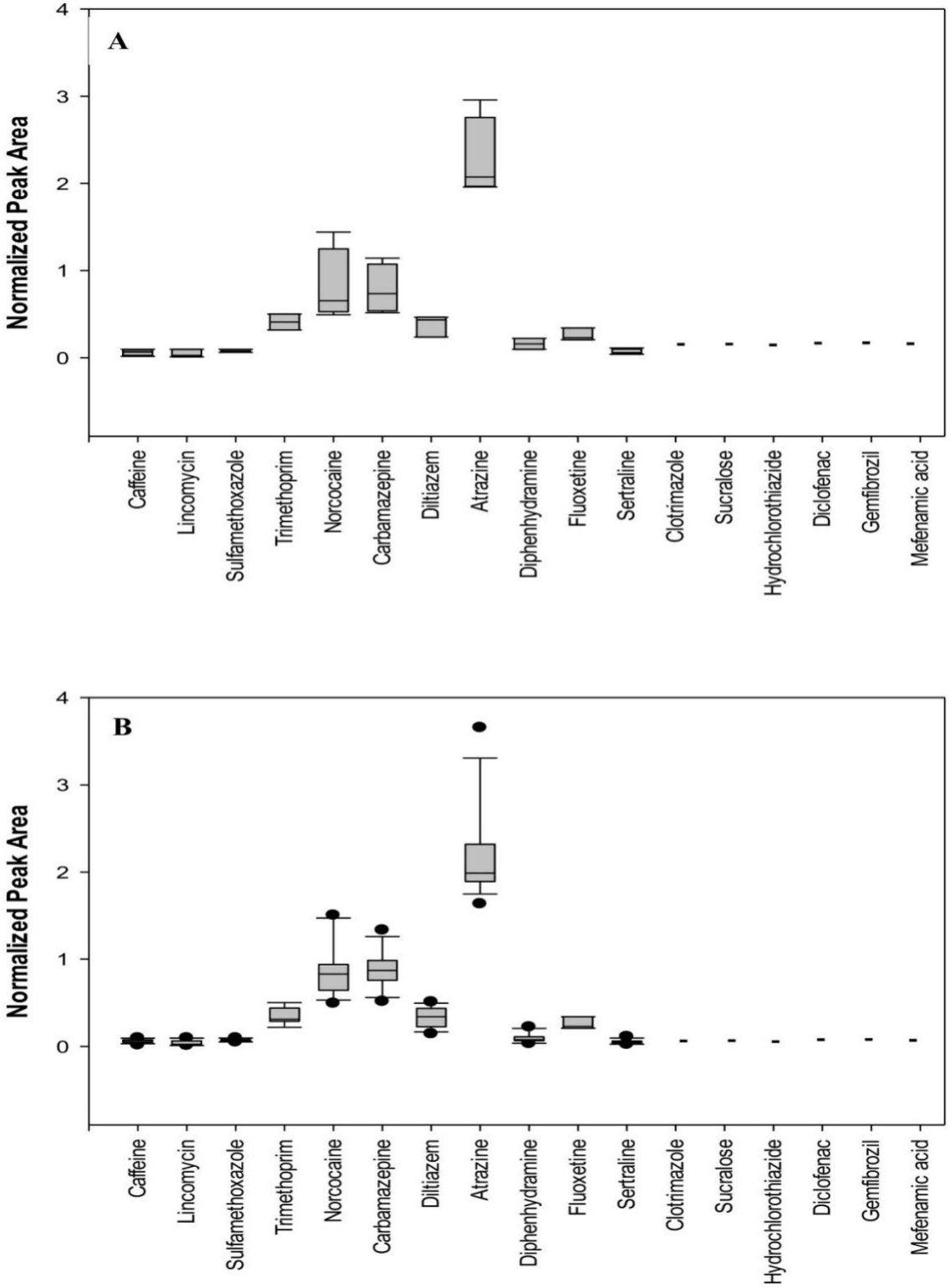

Figure 2.5 Normalized variation in peak area of the identified QC compounds within the same day (n=4) and over 3 consecutive days (n=14). A) intraday (top) and B) interday (bottom). (-) not normalized due to non-detect of the internal standard sucralose D6.

Normalizing the peak area for interday precision resulted in three of the previous four compounds (sulfamethoxazole, carbamazepine and atrazine) having a RSD ≤ 30%, except caffeine which had a RSD of 33.4% and fell within the 30% to 50% range. Trimethoprim improved and had a RSD of 28.4% (≤ 30%). Lincomycin still had a RSD ≥ 75%. Most of the compounds had a RSD between 30 to 50%. Overall, normalizing did not improve the outcome for interday analysis as well. The interday precision results for normalized peak area are shown in Figure 2.5B. Verkh *et. al*. used a mixture of 32 internal standards in their non-targeted analysis of dissolved organic matter in wastewater treatment to evaluate matrix suppression and instrument variation between injections and found that normalizing of the spectra lead to worse results in their replicates versus the unaltered spectra (Verkh et al., 2018). This was also observed by Nürenberg *et. al* (Nürenberg et al., 2015).

2.3.3 RT prediction Model: False positive reduction

The developed non-targeted workflow still needed some manual post-processing steps, which were accomplished by the use of appropriate filters based on the seven golden rules (Kind and Fiehn, 2007), prediction of retention and confirmation of peak performance. In addition to accuracy, precision, repeatability and reproducibility, we evaluated the selectivity of our NTA method in terms of the ability to reduce false positives. Therefore, we created a simple RT vs log K_{ow} model as shown in Figure 2.6, to provide a better understanding of how compounds are being retained and eluted according to their log K_{ow}. We expected less polar compounds to be retained more and elute at a later time with a C18 column. Based on this, we used this model to help restrict the massive amounts

of generated data and reduce false positives. The developed RT vs log K_{ow} model was applied to very complex mixtures in which 3 different mixtures (A, B and C) containing 95, 185 and 365 compounds respectively were analyzed (Ulrich et al., 2019). These complex samples were provided by the Environmental Protection Agency (EPA) as part of the EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) project and the analysis was initially performed blinded. After CD data processing, thousands of features were generated with our NTA workflow. Using the developed RT vs log K_{ow} model based on the QC mixtures, we were able to restrict the massive amount of data and greatly reduce false positives, expressed as false positive rate (as shown in Table 2.4).

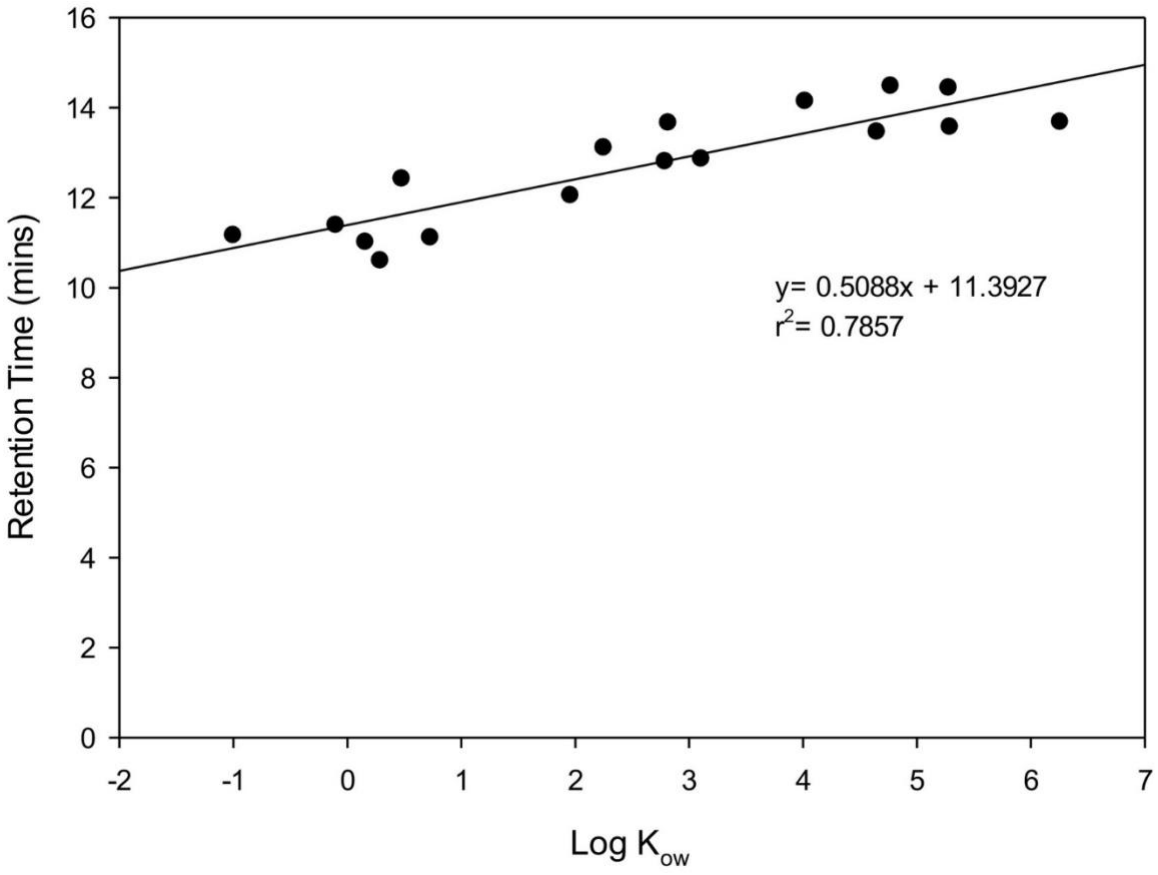

Figure 2.6 RT vs Log Kow model based on the QC mixtures for non-target analysis.

Table 2.4 Data post-processing showing false positive rate before and after QC based on the use of our RT prediction model.

	Complex Mixtures		
	A	B	C
Before QC	4753	7943	10788
After QC	215	275	254
Actual	95	185	365
False positive rate after QC	51.2%	50.9%	50.6%
False positive rate before QC	100%	100%	100%

False positive rate was calculated based on the following equation:

False positive rate = $\frac{false\ positives}{false\ positives + true\ negatives}$, where false positives are falsely identified as being present when it was not and true negatives are not present and correctly rejected (Fawcett, 2006). The developed RT vs log K_{ow} model based on the QC reduced false positives by 48.8%, 49.1% and 49.4% for each of the complex mixtures A, B and C respectively for an average false positive reduction of 49.1%. The results showed that unattended non-targeted analysis would lead to double of the compounds being wrongly identified in the samples, therefore, the use of QC samples has improved the quality of the data generated.

2.4 CONCLUSIONS

Commercially available software tools, such as Compound Discoverer, are making good progress in directing and automating non-targeted analysis, but there is still need of

additional post-processing steps and the assurance of quality of the data being generated. In this study, we evaluated the performance of analytical methodologies such as accuracy, precision (repeatability and reproducibility) and selectivity for non-target analysis in an in-house QC mixture prepared in LC-MS water and analyzed by online SPE coupled to UHPLC-ESI-HRMS. Based on the results, it is proposed that a precision of RSD ≤ 50% and accuracy of ≥ 70%, be an acceptable benchmark for non-target analysis. From the results, the use of just a single internal standard may not be enough to account for instrumental variations of a wide range of compounds, since no significant difference was observed by the normalizing of the data. The use of multiple internal standards that spans a wide range of compounds, covering not only a large range of classes but also ionization efficiencies and polarities, should be explored in future studies. The use of a simple retention time prediction model greatly helps in reducing the massive amounts of data generated for non-target analysis as well as reduced potential false positives.

2.5 ACKNOWLEDGMENTS

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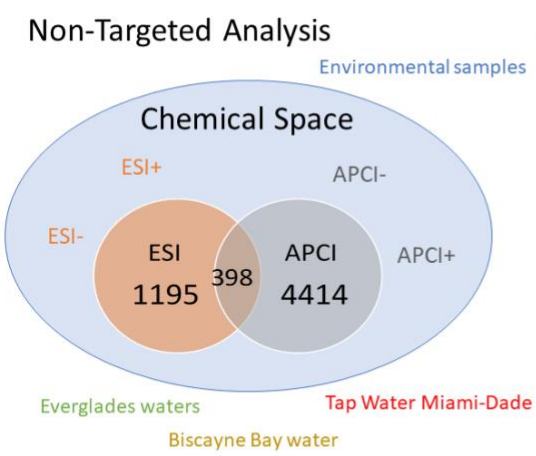
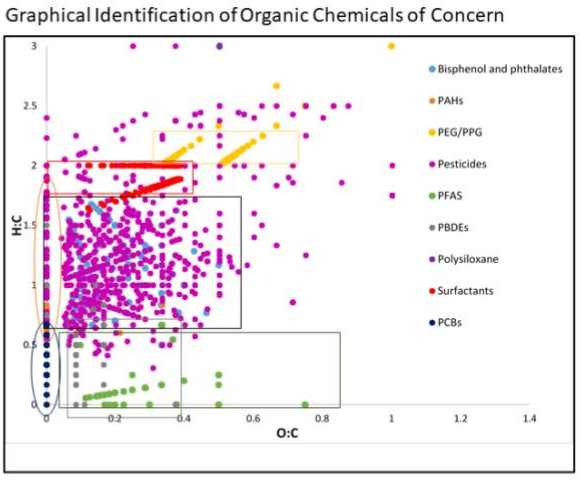
CHAPTER 3 COMPARISON OF DIFFERENT IONIZATION SOURCES IN THE DETECTION AND IDENTIFICATION OF ORGANIC CONTAMINANTS IN ENVIRONMENTAL WATERS BY NON-TARGETED ANALYSIS





3.1 INTRODUCTION

The advances in the field of high-resolution mass spectrometry offers great sensitivity and resolution, which coupled to ultra high-performance liquid chromatography represents a powerful tool for the separation and detection of a very large number of organic contaminants. At present, the most commonly used ionization sources in liquid chromatography with tandem mass spectrometry (LC-MS/MS) are atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). ESI and APCI sources are both soft ionization techniques. The ionization process associated with APCI is considered more efficient and energetic than ESI, where reactions in the gas phase may generate more fragment ions relative to the parent ion (Manheim et al., 2020). Although ESI is the most applied technique for polar and higher molecular weight compounds, APCI has shown to excel over ESI in the ionization of thermally stable polar and non-polar compounds (Cheng et al., 2015). Also, higher flow rates are more compatible in APCI than in ESI, which presents optimum sensitivity at lower flow rat es resulting in limitations for some compounds presenting lower sensitivity (Hanold et al., 2004). APCI has been reported to be more sensitive than ESI for triazines, phenylurea herbicides, organochlorine pesticides, polyaromatic hydrocarbons (PAH) and biphenols (Eichman Jr et al., 2017; Ghislain et al., 2012; Quinete et al., 2013; Thurman et al., 2001). In addition, APCI is considered less susceptible to matrix interferences and signal suppression compared to ESI (Dams et al., 2003; Duncan et al., 2015; Souverain et al., 2004). For certain classes of compounds that are traditionally very difficult to ionize or tend to show low sensitivity in LC-MS/MS, APCI has provided increased access to these compounds, being considered a complementary technique to ESI (Quinete et al., 2013).

The field of non-targeted analysis (NTA) is gaining increased attention, being applied in the fields of metabolomics (Di Guida et al., 2016), exposomics (Getzinger and Ferguson, 2020; Sobus et al., 2018), food safety (Knolhoff et al., 2016) and environmental analysis and monitoring (Hollender et al., 2017; Rager et al., 2016). A recent study has shown the valuable potential of NTA in which a previously unknown tire rubber derivative, [N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine]-quinone (6PPD-quinone), was identified by NTA as the chemical responsible for the annual mortality in coho salmon in U.S. Pacific Northwest , a phenomenon that has persisted for decades threatening conservation efforts (Tian et al., 2021b). The use of NTA has the potential to detect analytes of concern that would have been otherwise overlooked by target analysis and suspect screening approaches.

The majority of published NTA work is performed by high-resolution mass spectrometry (HRMS) coupled to an electrospray ionization (ESI) source. However, ESI in positive mode often produces cationic adducts such as sodium, ammonium and potassium adducts (Hanold et al., 2004; Lee et al., 2015), whereas APCI positive mode typically favors and produces the protonated ion $[M + H]^+$ due to its difference in ionization mechanisms and access to gas phase reactions (Thurman et al., 2001). This preferential formation of $[M + H]^+$ ions by APCI technique might constitute a key advantage for its use in NTA approaches, as the production of adducts may interfere with the convoluted NTA identification process, leading to increased false identification (false positives). In this study, environmental water samples (surface and tap waters) collected in South Florida were analyzed with both ionization techniques to characterize and fingerprint different water sources with the specific purpose of point source tracing. In addition, as part of the

interlaboratory study “Environmental Protection Agency’s (EPA) non-targeted analysis collaborative trial” (ENTACT) (Sobus et al., 2018; Ulrich et al., 2019), samples obtained were analyzed by both ESI and APCI under similar conditions to compare the different ionization method in a situation where the identity of chemicals were known, exploring whether APCI has complementary or superior performance than that of ESI for the purpose of NTA.

3.2 MATERIALS AND METHODS

3.2.1 Chemicals and reagents

The chemical list and descriptions are the same as those used by (Ng et al., 2020) in which all chemical and reagents used were of high purity. The reagents used (water, methanol, acetonitrile and formic acid) were Optima LC/MS grade from Fisher Scientific (Fair Lawn, NJ, USA) and the standards (sucralose, hydrochlorothiazide, diclofenac sodium salt, gemfibrozil, mefenamic acid, caffeine, lincomycin, sulfamethoxazole, trimethoprim, norcocaine, carbamazepine, diltiazem hydrochloride, atrazine, diphenhydramine hydrochloride, fluoxetine hydrochloride, sertraline hydrochloride and clotrimazole) were of high purity (>90%) from various commercial vendors (Sigma-Aldrich, AK Scientific, MP Biomedicals, Cerilliant). A group of native standards with different polarities (listed in Table 3.1) were combined to create quality control (QC) mixtures in LCMS grade methanol as described by (Ng et al., 2020). Working solutions of the QC mixtures were prepared at a concentration of 200 ng/mL in methanol, where 20 µL were diluted in 10.50 mL of LC-MS grade water (final concentration of 381 ng/L) for online solid phase extraction (SPE) procedure.

Table 3.1 List of quality control compounds and their respective log K_{ow}, molecular formula, monoisotopic mass and monitored ions. Adapted from Ng 2020.

Compound	Log K_{ow}	Molecular formula	Monoisotopic mass	Monitored ions	Retention time (min)
Sucralose	-1.00	$C_{12}H_{19}Cl_3O_8$	396.0146	395.0073[b]	11.16
Hydrochlorothiazide	-0.10	$C_7H_8ClN_3O_4S_2$	296.9645	295.9572[b]	11.39
Caffeine	0.16	$C_8H_{10}N_4O_2$	194.0804	195.0877[a]	11.01
Lincomycin	0.29	$C_{18}H_{34}N_2O_6S$	406.2137	407.2210[a]	10.60
Sulfamethoxazole	0.48	$C_{10}H_{11}N_3O_3S$	253.0521	254.0594[a]	12.42
Trimethoprim	0.73	$C_{14}H_{18}N_4O_3$	290.1379	291.1452[a]	11.11
Norcocaine	1.96	$C_{16}H_{19}NO_4$	289.1314	290.1387[a]	12.05
Carbamazepine	2.25	$C_{15}H_{12}N_2O$	236.0950	237.1022[a]	13.11
Diltiazem	2.79	$C_{22}H_{26}N_2O_4S$	414.1613	415.1686[a]	12.80
Atrazine	2.82	$C_8H_{14}ClN_5$	215.0938	216.1010[a]	13.66
Diphenhydramine	3.11	$C_{17}H_{21}NO$	255.1623	256.1696[a]	12.86
Diclofenac	4.02	$C_{14}H_{11}Cl_2NO_2$	295.0167	294.0094[b]	14.14
Fluoxetine	4.65	$C_{17}H_{18}F_3NO$	309.1341	310.1413[a]	13.46
Gemfibrozil	4.77	$C_{15}H_{22}O_3$	250.1569	249.1496[b]	14.48
Mefenamic acid	5.28	$C_{15}H_{15}NO_2$	241.1103	240.1030[b]	14.44
Sertraline	5.29	$C_{17}H_{17}Cl_2N$	305.0738	306.0811[a]	13.57
Clotrimazole	6.26	$C_{22}H_{17}ClN_2$	344.1080	345.1153[a]	13.68

[a]Ions were monitored in ESI positive and APCI (70.6%), [b]Ions were monitored in ESI and APCI negative.

3.2.2 Sample collection

The sampling locations were chosen based on the ecological importance of each water source and the different unique water body representation in South Florida (Ng et al., 2021). The coordinates for each sampling location are presented in Table 3.2. The Everglades National Park in South Florida is the world's largest subtropical wilderness and spans over 6102 km^2, including marine, estuarine and freshwater environments (Cui et al., 2020; Wiley and Simpfendorfer, 2007). Whereas Biscayne Bay is a sub-tropical estuary located in the southeastern coast of Florida that supports a vast variety of fauna and is an important part of the economic and recreational life of South Florida (Ng et al., 2021). The source of potable water in Miami-Dade County, FL is groundwater from the Biscayne Aquifer that has gone through rigorous treatment processes to meet stringent criteria's set by the EPA for human consumption and use.

Table 3.2 Coordinates of sample locations from the Everglades National Park, Biscayne Bay and its related canals and tap water from Miami-Dade County.

Location	Sampling sites	Latitude	Longitude
Everglades National Park	Airboat trail	25°45'44.5"N	80°46'09.4"W
	Site 1	25°45'44.4"N	80°43'37.0"W
	Site 3	25°45'44.4"N	80°40'54.5"W
Biscayne Bay and its related canals	Royal Galdes Canal	25°55'44.05"N	80°9'6.39"W
	Biscayne Canal 8	25°52'16.16"N	80°10'36.96"W
	Seybold Canal	25°47'3.87"N	80°12'38.39"W
Tap Water	Aventura	25°57'28.872"N	80°11'10.068"W
	Coral Gables	25°44'58.128"N	80°13'58.404"W
	Miami	25°36'58.68"N	80°19'29.784"W

Although tap water has gone through treatment processes, it has been found to still contain contaminants of concern (Rosario-Ortiz et al., 2016; Sharma and Bhattacharya, 2017). Water samples from the Everglades National Park which is a freshwater marsh (n=3) and represents a pristine environment, Biscayne Bay and related canals (n=3) (links) which represents a coastal lagoon and its associated urban environment and potable tap water (end-point) from Miami-Dade County (n=3) were collected in 2017 using 500 mL Teflon bottles and transported to the lab in a cooler with ice. Samples were kept refrigerated at 4°C until analysis. Before collection, Teflon bottles were thoroughly washed in triplicates with Optima LC/MS grade methanol, acetonitrile, acetone, hexane and methylene chloride and thoroughly rinsed with deionized water. In addition to the collected environmental samples, EPA's ENTACT samples (Sobus et al., 2018; Ulrich et al., 2019) were analyzed. Ten ENTACT samples were made from the EPA's ToxCast chemical library and contained either 95, 185 or 365 chemical substances each in dimethyl sulfoxide (DMSO) (Singh et al., 2020).

3.2.3 Quality control

While there are no established standard quality assurance/quality control (QA/QC) guidelines currently for NTA, a few measures were taken to ensure quality of data as previously outlined by (Ng et al., 2020). Prior to every analysis, the instrument (Q-Exactive Orbitrap, Thermo Scientific, USA) was calibrated in both positive and negative mode using Pierce LTQ ESI positive and negative ion calibration solutions (Thermo Scientific, USA) and mass accuracy checked to ensure that it is below a mass tolerance of 5 ppm. In addition, mass accuracy was maintained throughout each analytical batch by using an insource lock

mass of 391.2843 (diisooctyl phthalate) to correct for any mass drift that may occur. A part of the quality control routine, blank samples consisting of LC/MS water and QC mixtures containing 17 selected compounds with varying log K_{ow} were analyzed daily in the beginning of the run and after every 5 samples analyzed (each sample was analyzed by full scan positive mode, followed by data dependent MS/MS (dd-MS/MS) and full scan negative mode, followed by dd-MS/MS) to check for background contamination and to ensure instrument and analytical performance. Variations in retention time (RT) and intensity were checked to identify analytical issues before, during and after analysis. Variations higher than 30% in RT or 40% in intensity would identify analytical issues which could be corrected, and analysis would be re-done. Variations in RT were less than RSD $\leq$ 10%, while in intensity were <30% for APCI and <40% for ESI as shown for some of the QC compounds in Figure 3.1 below.

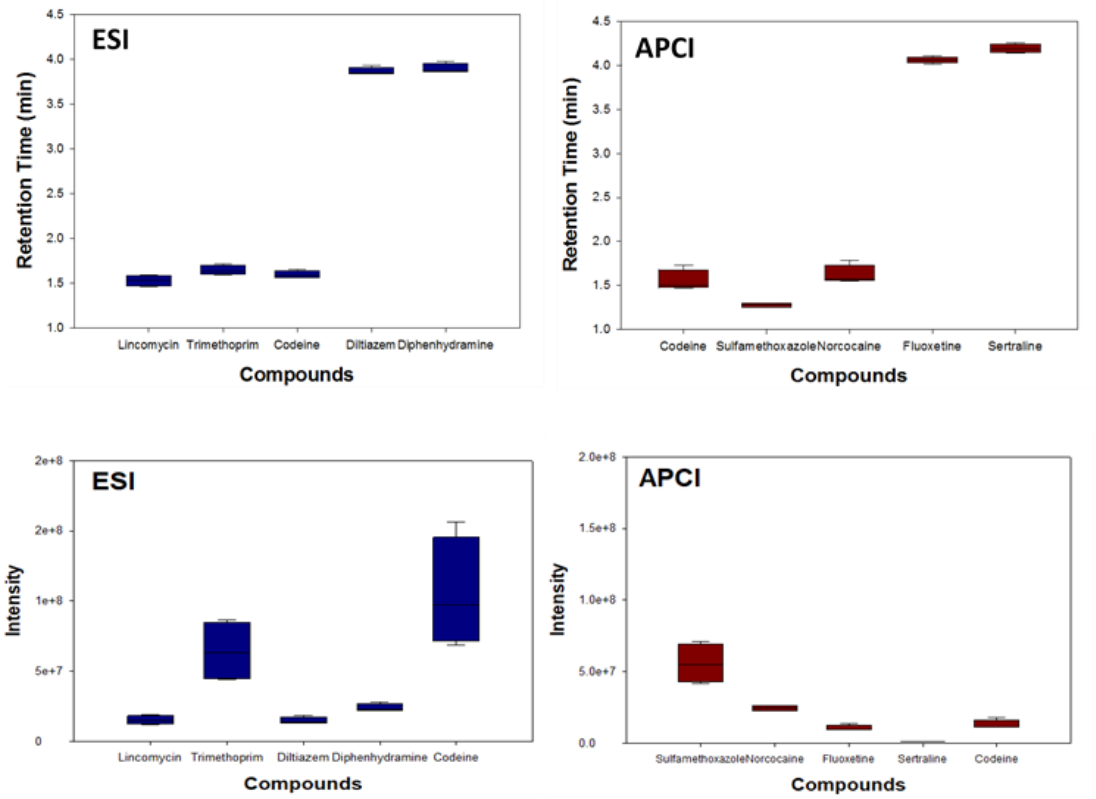


Figure 3.1 Variation in retention time and intensity for some of the quality control compounds by ESI and APCI positive mode analysis.

3.2.4 High-resolution mass spectrometry analysis

Environmental water samples analysis were analyzed by online-solid phase extraction (SPE), in which 10 mL of pre-filtered samples were loaded in the SPE column following the methodology previously published by Ng et al., 2020 and summarized in Table 3.3 and Table 3.4 in the supporting information.

Table 3.3 Gradient of pump 2 for the online SPE pre-concentration step.

Pump 2 – Online SPE						
	Time	A%	B%	C%	D%	µL/min
0	0.00	98.0	0.0	2.0	0.0	200.0
1	0.10	98.0	0.0	2.0	0.0	2500.0
2	4.20	98.0	0.0	2.0	0.0	2500.0
3	4.50	0.0	0.0	100.0	0.0	1000.0
4	6.50	0.0	0.0	100.0	0.0	1000.0
5	7.00	10.0	90.0	0.0	0.0	1000.0
6	8.00	10.0	90.0	0.0	0.0	1000.0
7	8.90	98.0	0.0	2.0	0.0	1000.0
8	15.00	98.0	0.0	2.0	0.0	1000.0

A: water, B: methanol, C: acetonitrile, D: 0.1% formic acid

Table 3.4 Gradient of pump 1 for the elution and separation step by the analytical column by online SPE.

Pump 1 – Analytical column						
	Time	A%	B%	C%	D%	µL/min
0	0.00	0.0	0.0	2.0	98.0	250.0
1	4.20	0.0	0.0	2.0	98.0	250.0
2	10.00	0.0	0.0	60.0	40.0	250.0
3	12.00	0.0	0.0	100.0	0.0	250.0
4	15.00	0.0	0.0	100.0	0.0	250.0

A: water, B: methanol, C: acetonitrile, D: 0.1% formic acid

The ENTACT samples, consisting of mixtures of chemicals containing up to 365 compounds prepared in dimethyl sulfoxide (DMSO) at a concentration of approximately 0.05 mM per chemical, were diluted (10X) with LCMS grade methanol prior to analysis by direct injection (20 µL of sample). The direct injection analytical gradient used for separation is shown in Table 3.5.

Table 3.5 Gradient for the elution and separation by the analytical column by direct injection.

Analytical column						
	Time	A%	B%	C%	D%	µL/min
0	0.00	0.0	0.0	2.0	98.0	250.0
1	1.50	0.0	0.0	60.0	40.0	250.0
2	3.00	0.0	0.0	100.0	0.0	250.0
3	10.00	0.0	0.0	100.0	0.0	250.0
4	11.00	0.0	0.0	2.0	98.0	250.0

A: water, B: methanol, C: acetonitrile, D: 0.1% formic acid

The heated electrospray ionization (HESI) source (Thermo Scientific, USA) was used, at a spray voltage of 5.00 kV for both positive and negative mode, a capillary temperature of 350 ˚C, auxiliary gas heater temperature of 250 ˚C, a sheath gas flow of 30 arbitrary units and auxiliary gas flow of 2 arbitrary units. Samples were also analyzed with an APCI source with a corona discharge current of 5.00 µA and the same ionization source parameters as that of ESI; a capillary temperature of 350 ˚C, auxiliary gas heater temperature of 250 ˚C, a sheath gas flow of 30 arbitrary units and auxiliary gas flow of 2 arbitrary units. Blanks (LC-MS water), QC samples, ENTACT and environmental samples

were analyzed in full scan positive mode (scan range 100.0 to 800.0 m/z) at a resolution of 140,000, followed by data dependent MS/MS (dd-MS/MS) with a normalized collision energy of 30 at a resolution of 35,000. This procedure was repeated for the negative mode. This was done for both ESI and APCI.

The ENTACT samples were analyzed by direct injection, this means without the SPE process, since it consisted of a complex mixture of chemicals (which identity were initially unknown) and the online-SPE process could potentially lead to the elimination of compounds of interest. This analysis was first conducted as a blinded study to prevent bias and then reevaluated unblinded. The analysis of environmental water samples was done by online-SPE as a cleanup and preconcentration step, due to the complex nature of the matrix and the fact that environmental contaminants are often found in low concentration.

3.2.5 Data post-processing

Data post-processing was done following previously published work using Compound Discoverer (CD) 3.0 (Thermo Scientific, USA) and some additional post-processing steps utilizing retention time vs log K_{ow} model, filters based on the seven golden rules, confirmation of peak performance and isotopic patterns (Ng et al., 2020). The databases searched for this study were Chemspider, MzCloud, MzVault, DrugBank, EAWAG Biocatalysis/Biodegradation, EPA Toxcast and DSSTox, ACToR: (Aggregated Computational Toxicology Resource) and FDA (Food and Drug Administration) UNII – NLM databases. In addition to the searched databases, the following mass lists included in CD were searched: EFS HRAM Compound Database, Endogenous Metabolites database 4400 compounds and Extractables and Leachables HRAM Compound Database.

Background subtraction was done using the blank samples. The peak picking parameters were performed using an intensity tolerance of 30% and a minimum peak intensity of 100,000. The grouping and merging of features were done at a mass tolerance of 5 ppm and a retention time (RT) tolerance of 0.1 min, where a feature is defined by a chromatographic peak with an exact mass and its retention time (Nürenberg et al., 2019; Singh et al., 2020). Elemental composition prediction and fragment matching was carried out with maximum element counts of C90, H190, Br3, Cl6, N10, O18, P3, and S5, a maximum ring double bond equivalents (RDBE) of 40, and maximum H/C of 3.5. Pattern matching was done with an intensity tolerance of 30%, intensity threshold of 0.1%, minimum spectral fit of 30% and a minimum pattern coverage of 80%. The mass tolerance for all compounds was less than 5 ppm and S/N of 3. Baseline subtraction is automatically performed by the software's algorithm using a blank. After the initial data processing through CD, a list of features are obtained and additional manual processing was carried out to further reduce features that did not meet additional quality control criteria such as the peak area of the sample must be three times higher than that of the blank (S/N > 3), chromatographic peaks were individually checked to avoid noise integration and the retention time of the tentative candidates must be within 0.5 min based on the developed RT vs Log K_{ow} model (Ng et al., 2020).

3.3 RESULTS AND DISCUSSION

3.3.1 ESI vs APCI for the detection and identification of environmental organic contaminants in South Florida water systems

To evaluate both ionization methods in environmental samples for the

characterization and fingerprinting of different water sources to be used for point source tracing, surface water from the Everglades National Park, Biscayne Bay and its related canals and tap water from Miami-Dade County in South Florida were collected and analyzed. Overall, only 398 features were detected by both ESI and APCI, while 1195 features were detected only by ESI and 4414 by APCI in all samples analyzed (n=9) (Figure 3.2).

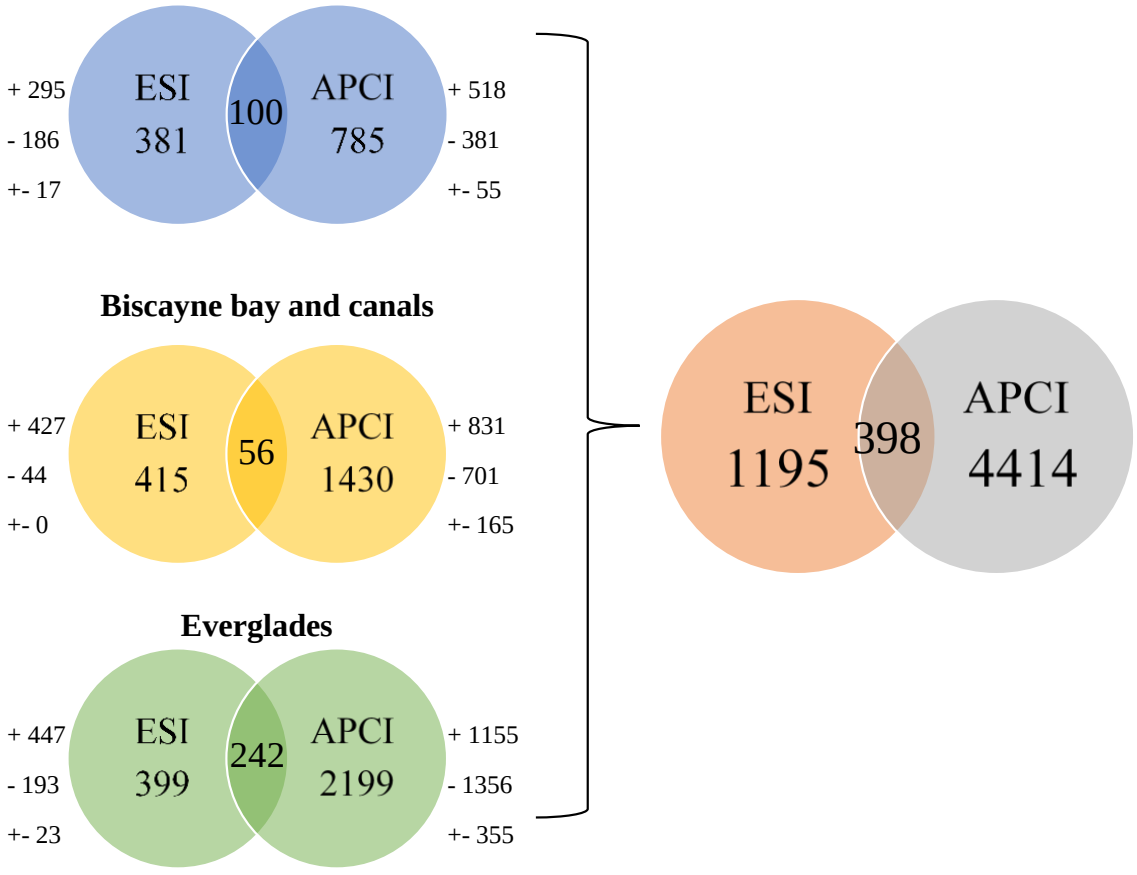


Figure 3.2 Venn diagram of overall unique features with at least a tentative candidate detected by ESI and APCI of different sources of water.

Considering features with MS^2 matches only (50% match or greater library match in mzCloud, mzVault or mzCloud best similarity match), the Everglades National Park had 1341 features detected, while 1176 features were detected in Biscayne Bay and its related canals and 807 features were detected in tap waters, for both ESI and APCI combined. Of these detected features, 53% (709) were unique only to samples from the Everglades National Park, 52% (617) were unique to Biscayne Bay and its related canals and 44% (358) were unique only to tap water samples (Figure 3.3).

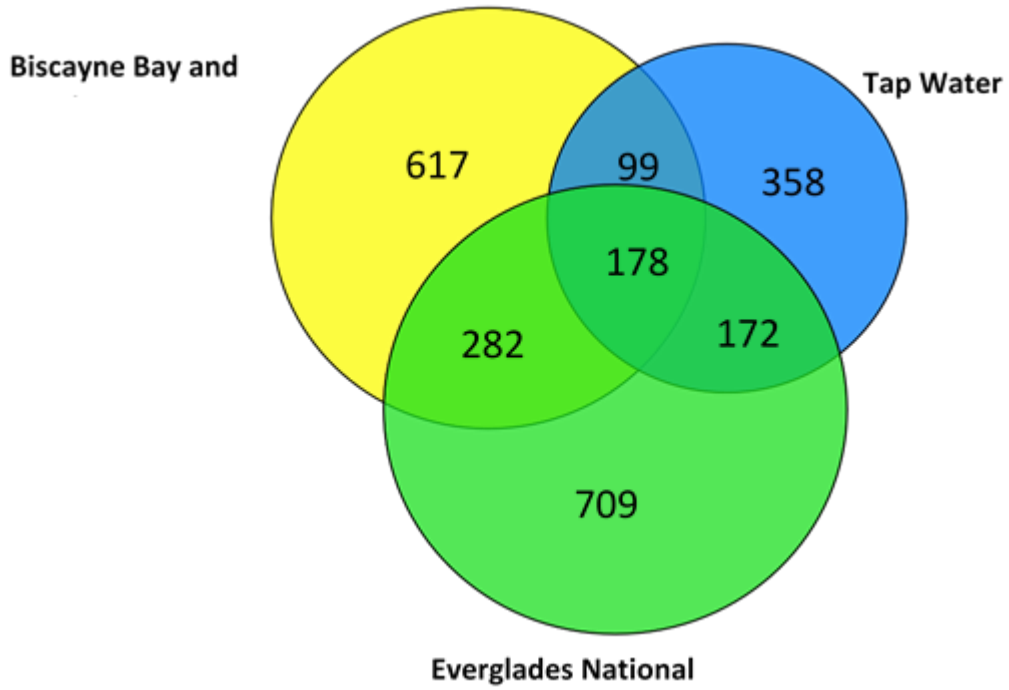


Figure 3.3 Venn diagram of unique features detected using both ESI and APCI combined for different sources of water (Everglades National Park, Biscayne Bay and its related canals and tap water) and their overlap.

APCI had a greater detection of features than ESI. This difference is more evident for the surface waters from the Everglades National Park than from Biscayne Bay and its

related canals and tap water, which can be due to the higher amounts of natural organic matter in the Everglades National Park, followed by Biscayne Bay and its related canals and tap water as well as the ability of APCI to ionize a larger number of compounds. We were able to detect and identify common chemical features (features that were present in all 3 samples analyzed for each sample type) among sample types (Everglades National Park, Biscayne Bay and canals, and tap water). Tap waters were found to have 16 chemical features that were ubiquitous in all samples with 6 of these being only identified by ESI and 10 by APCI. Compounds identified in tap water were classified as 25% pharmaceuticals, 19% pesticides, 25% natural products, 13% food additive, fragrance and dye, 12% multicategory (multipurpose use) and 6% other (could not be classified or classification unknown), with no industrial compounds being detected. Interestingly, 2-hydroxyatrazine and 2-hydroxypropazine, two different pesticide transformation products were detected in all tap water samples analyzed, demonstrating that they are not fully removed by drinking water treatment plants (Guillon et al., 2018).

Water samples from the Everglades National Park were found to have 33 chemical features that were ubiquitous throughout the samples, 12 of which were identifiable by ESI and 21 by APCI. The unique composition makeup of chemical classes found in each water source is shown in Figure 3.4.

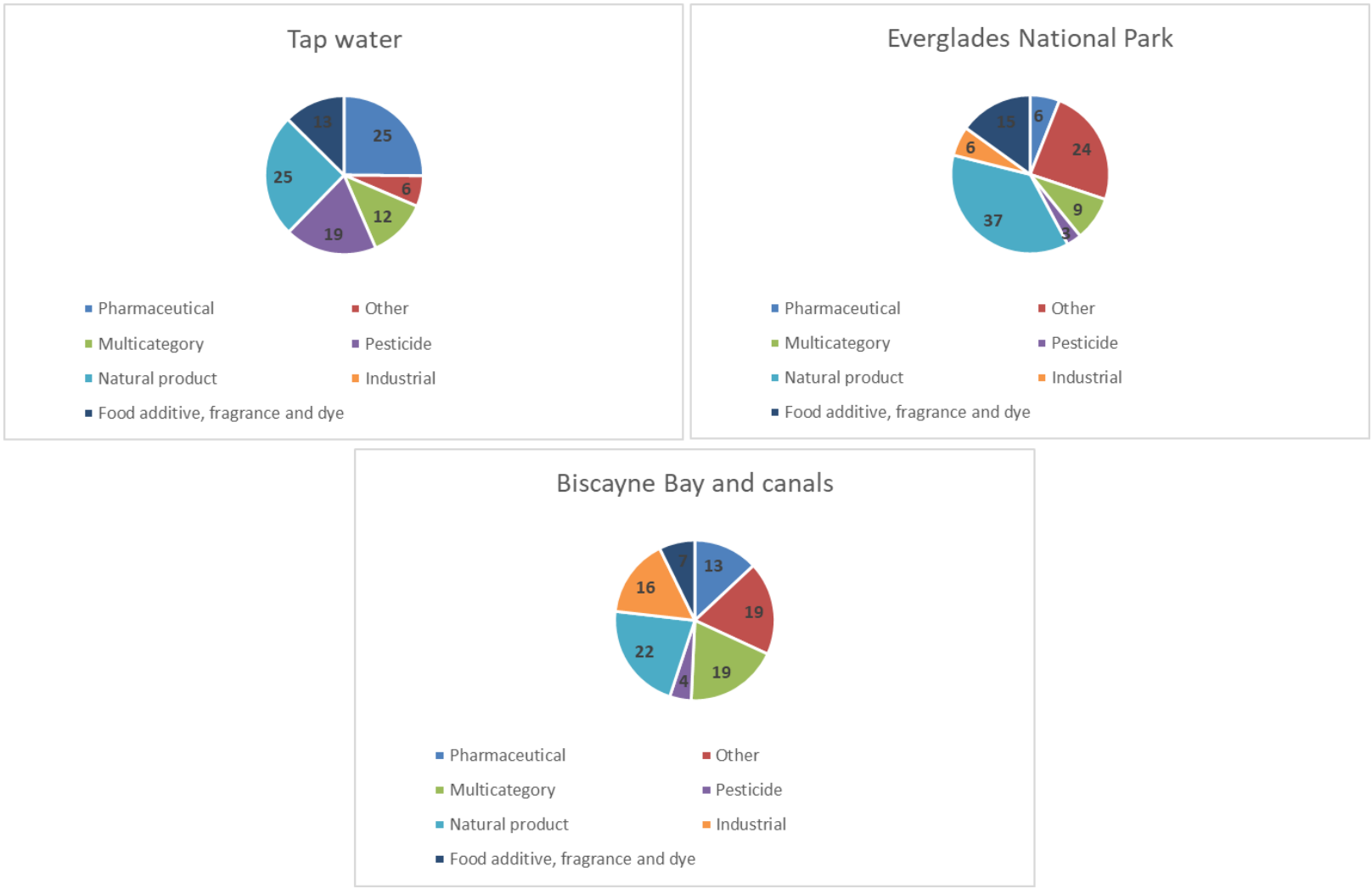


Figure 3.4 Composition makeup of chemical classes found in each water source.

Tap water had the highest prevalence of pharmaceuticals (25%) and pesticides (19%) compared to the Biscayne Bay water and its related canals (13% and 4%, respectively) and the Everglades National Park (6% and 3%, respectively). However, there were no industrial compounds detected for tap water, while they were detected in both Everglades National Park (6%) and Biscayne Bay and its related canals (16%). Tap waters (13%) and waters from the Everglades National Park (15%) had approximately twice the amount of food additive, fragrance and dye than Biscayne Bay and its related canals (7%). These identified features are listed in Table 3.6.

Table 3.6 Identified features (with MS2 database matches, Schymanski confidence level 2b) in each source of water by ESI and APCI.

Water Source	Chemical feature	RT (min)	Molecular weight	Ionization method	Classification
Tap water	(+)-brefeldin A	13.38	280.1680	ESI	Pharmaceutical
Tap water	[hydroxy]3beta_16alpha-dihydroxy-5-androsten-17-one	13.59	304.2044	ESI	Natural product
Tap water	Arenediol	13.52	112.0526	ESI	Other
Tap water	Benzoyl-y-tropeine	13.53	245.1412	ESI	Pharmaceutical
Tap water	Fenuron	8.84	164.0950	ESI	Pesticide
Tap water	Tetrahydropteridine	13.47	136.0745	ESI	Natural product
Tap water	17α-Hydroxyprogesterone	13.74	330.2206	APCI	Natural product
Tap water	2-Hydroxyatrazine	10.15	197.1273	APCI	Pesticide
Tap water	2-Hydroxypropazine	10.63	211.1433	APCI	Pesticide

Tap water	3-Methyldioxyindole	9.49	163.0630	APCI	Natural product
Tap water	4-(1,4-Dioxaspiro[4.5]dec-8-yl)cyclohexanone	13.04	238.1571	APCI	Food additive, fragrance and dye
Tap water	Arecoline	6.54	155.0943	APCI	Pharmaceutical
Tap water	BHEB (2,6-Di-tert-butyl-4-ethylphenol)	14.00	234.1976	APCI	Multicategory
Tap water	Dodecylsuccinic Anhydride	14.18	268.2031	APCI	Food additive, fragrance and dye
Tap water	PEG	9.92	414.2454	APCI	Multicategory
Tap water	Phenacetin	10.02	179.0940	APCI	Pharmaceutical
Everglades National Park	(2,4-Diamino-6-pteridinyl)methanol	6.30	192.0760	ESI	Other
Everglades National Park	2-Ethylhexyl 4-(dimethylamino)benzoate	12.76	277.2035	ESI	Pharmaceutical
Everglades National Park	3-Hydroxycotinine(3HC)	8.77	192.0898	ESI	Natural product
Everglades National Park	4-(1-Amino-2-methylpropyl)-1,6-	10.90	183.1622	ESI	Other

	heptadien-4-ol				
Everglades National Park	4,5-Dioxo-4,5-dihydro-3-furancarbaldehyde	6.07	125.9953	ESI	Food additive, fragrance and dye
Everglades National Park	Dimethocaine	11.07	278.1992	ESI	Pharmaceutical
Everglades National Park	D-Pipecolicacid	6.00	129.0790	ESI	Natural product
Everglades National Park	Geranyl isopentanoate	13.57	238.1928	ESI	Food additive, fragrance and dye
Everglades National Park	Methyl 2-chloro-1,3-oxazole-4-carboxylate	6.49	160.9877	ESI	Other
Everglades National Park	N-benzyl-N,N'-dimethylamine	9.00	135.1048	ESI	Industrial
Everglades National Park	UNII:13W9041KWU (13(S)-HPODE methyl ester)	13.91	326.2450	ESI	Natural Product
Everglades National Park	UNII:FL8S7F2JJQ ((R)-3-Hydroxytetradecanoic acid)	14.31	244.2038	ESI	Natural Product
Everglades National Park	(+/-)12(13)-DiHOME	14.02	296.2359	APCI	Natural product
Everglades National Park	13(S)-HpOTrE	14.37	292.2049	APCI	Natural product

Everglades National Park	18K00A531C	8.45	162.0425	APCI	Pesticide
Everglades National Park	2,6-Di-tert-butylbenzoquinone	12.92	220.1465	APCI	Multicategory
Everglades National Park	2-(8-Hydroxy-4a,8-dimethyldecahydro-2-naphthalenyl)acrylic acid	13.35	252.1732	APCI	Natural product
Everglades National Park	2-[(2S,4aR,8aS)-2-Hydroxy-4a-methyl-8-methylenedecahydro-2-naphthalenyl]acrylic acid	14.49	250.1572	APCI	Other
Everglades National Park	2-Hydroxymyristic acid	14.34	244.2043	APCI	Natural product
Everglades National Park	2-Hydroxyphenethylamine	10.92	137.0840	APCI	Other
Everglades National Park	20-Hydroxy-(5Z,8Z,11Z,14Z)-eicosatetraenoic acid	13.62	320.2360	APCI	Natural product
Everglades National Park	3-Hydroxy-4-(2-hydroxy-6-	13.17	266.1529	APCI	Other

	methyl-2-heptanyl)benzoic acid				
Everglades National Park	3-Methyldioxyindole	10.70	163.0628	APCI	Food additive, fragrance and dye
Everglades National Park	3,5-Di-tert-butyl-4-hydroxybenzaldehyde	14.06	234.1619	APCI	Multicategory
Everglades National Park	3,5-di-tert-Butyl-4-hydroxybenzyl alcohol	14.28	236.1779	APCI	Food additive, fragrance and dye
Everglades National Park	4-Dodecylbenzenesulfonic acid	13.51	326.1925	APCI	Industrial
Everglades National Park	BJ0YZC8ZY8	12.85	236.1421	APCI	Multicategory
Everglades National Park	Diethyl Terephthalate	10.89	222.0903	APCI	Food additive, fragrance and dye
Everglades National Park	Docosahexaenoic acid	13.45	318.2756	APCI	Multicategory
Everglades National Park	Methylfarnesoate	10.55	250.1929	APCI	Other

Everglades National Park	Oleic acid alkyne	15.08	278.2253	APCI	Natural product
Everglades National Park	Rishitin	12.45	222.1615	APCI	Natural product
Everglades National Park	Stearic acid	14.71	284.2723	APCI	Natural product
Biscayne Bay and canals	(R)-6-Hydroxynicotine	6.21	178.1108	ESI	Natural product
Biscayne Bay and canals	1-(1-Methyl-4-piperidinyl)piperazine	6.22	183.1736	ESI	Other
Biscayne Bay and canals	1-butylimidazole	6.50	124.1003	ESI	Food additive, fragrance and dye
Biscayne Bay and canals	1-Ethyl-4-(4-propylcyclohexyl)benzene	14.16	230.2032	ESI	Other
Biscayne Bay and canals	2,4-Dimethyl-1,3-diphosphete	6.18	115.9946	ESI	Other
Biscayne Bay and canals	2,8-Diazaspiro[4.5]decan-3-one	6.53	154.1107	ESI	Industrial (UV absorber)

Biscayne Bay and canals	3-(3,5-dimethyl-1H-1,2,4-triazol-1-yl)propan-1-amine	6.24	154.1219	ESI	Industrial (buffer)
Biscayne Bay and canals	3-Cyclopropyl-1-methyl-1H-pyrazol-5-amine	6.21	137.0954	ESI	Food additive, fragrance and dye
Biscayne Bay and canals	4-Trimethylammoniobutanal	11.28	129.1155	ESI	Natural product
Biscayne Bay and canals	6-(Morpholin-4-yl)pyrimidin-4-amine	15.14	180.1012	ESI	Other
Biscayne Bay and canals	Eplerenone	13.34	414.2042	ESI	Pharmaceutical
Biscayne Bay and canals	Mauve Factor Reagent	6.22	123.1050	ESI	Industrial
Biscayne Bay and canals	Minoxidil	6.21	209.1277	ESI	Pharmaceutical
Biscayne Bay and canals	Triethylenediamine	6.38	112.1004	ESI	Industrial

Biscayne Bay and canals	(4-tert-butylphenyl)acetic acid	14.27	192.1149	APCI	Pesticide
Biscayne Bay and canals	(S)-beta-Methylindolepyruvate	11.13	217.0738	APCI	Other
Biscayne Bay and canals	1,2-Diaminobenzene	8.85	108.0690	APCI	Multicategory
Biscayne Bay and canals	1-Benzyl-3-pyrrolidinecarboxylic acid	10.99	205.1100	APCI	Other
Biscayne Bay and canals	1-Stearoylglycerol	15.34	358.3076	APCI	Natural product
Biscayne Bay and canals	1-Vinyl-2-pyrrolidinone	8.87	111.0685	APCI	Multicategory
Biscayne Bay and canals	13(S)-HpOTrE	14.14	292.2050	APCI	Natural product
Biscayne Bay and canals	2,5-Dihydroxybenzaldehyde	8.84	138.0315	APCI	Multicategory
Biscayne Bay and canals	2,5-Dihydroxypyridine	8.85	111.0322	APCI	Other

Biscayne Bay and canals	2-(8-Hydroxy-4a,8-dimethyldecahydro-2-naphthalenyl)acrylic acid	13.81	252.1733	APCI	Natural product
Biscayne Bay and canals	2-[(2S,4aR,8aS)-2-Hydroxy-4a-methyl-8-methylenedecahydro-2-naphthalenyl]acrylic acid	14.08	250.1576	APCI	Other
Biscayne Bay and canals	2-Furoate	9.83	112.0162	APCI	Natural product
Biscayne Bay and canals	2-Methoxy-aniline	8.88	123.0685	APCI	Multicategory
Biscayne Bay and canals	2-Methoxyresorcinol	8.86	140.0471	APCI	Natural product
Biscayne Bay and canals	2-Methylnicotinamide	8.87	136.0635	APCI	Natural product
Biscayne Bay and canals	2-Oxooctadecanoic acid	15.37	298.2512	APCI	Natural product
Biscayne Bay and canals	2-Phenylcyclopropylamine	9.95	133.0892	APCI	Pharmaceutical

Biscayne Bay and canals	3-Hydroxypicolinicacid	9.83	139.0267	APCI	Industrial
Biscayne Bay and canals	3779MVZ8JX	10.23	274.1314	APCI	Other
Biscayne Bay and canals	4-(4-Nitrobenzyl)pyridine	10.03	214.0737	APCI	Industrial
Biscayne Bay and canals	4-Ethoxy ethylbenzoate	14.28	194.0939	APCI	Industrial
Biscayne Bay and canals	4-Hydroxymethylphenylhydrazine	9.92	138.0791	APCI	Other
Biscayne Bay and canals	4-Nitroacetophenone	8.96	165.0418	APCI	Pharmaceutical
Biscayne Bay and canals	4-Nitroaniline	8.85	138.0428	APCI	Multicategory
Biscayne Bay and canals	4-Nonylphenol	7.12	220.1824	APCI	Multicategory

Biscayne Bay and canals	6-Methylquinoline	10.20	143.0735	APCI	Food additive, fragrance and dyes
Biscayne Bay and canals	9-Nitrooleate	13.43	344.2666	APCI	Natural product
Biscayne Bay and canals	alpha-Santonin	11.98	246.1261	APCI	Multicategory
Biscayne Bay and canals	Arecoline	6.88	155.0945	APCI	Pharmaceutical
Biscayne Bay and canals	Benzaldehyde	13.37	106.0422	APCI	Multicategory
Biscayne Bay and canals	Columbianetin	11.10	246.0892	APCI	Natural product
Biscayne Bay and canals	Diethyl diisopropylphosphoramidoite	7.13	221.1541	APCI	Industrial
Biscayne Bay and canals	Diphenylamine	14.26	169.0888	APCI	Multicategory
Biscayne Bay and canals	Equol	11.10	242.0942	APCI	Natural product

Biscayne Bay and canals	Ethyl 2-ethoxy-3-(4-hydroxyphenyl)propanoate	13.32	238.1208	APCI	Multicategory
Biscayne Bay and canals	Ethyl 4-hydroxy-8-methyl-3-quinolinecarboxylate	11.67	231.0891	APCI	Food additive, fragrance and dyes
Biscayne Bay and canals	Ethyl paraben	10.88	166.0627	APCI	Multicategory
Biscayne Bay and canals	Hexadecanehydrazide	13.87	270.2667	APCI	Pharmaceutical (antimicrobial)
Biscayne Bay and canals	Imidazole-4-acetaldehyde	8.87	110.0479	APCI	Other
Biscayne Bay and canals	Indole-3-ethanol	10.01	161.0840	APCI	Natural product
Biscayne Bay and canals	Indoleamine	8.87	132.0687	APCI	Other
Biscayne Bay and canals	Isobornyl Acrylate	14.61	208.1462	APCI	Multicategory

Biscayne Bay and canals	Isoquinoline	9.83	129.0578	APCI	Multicategory
Biscayne Bay and canals	lrganox degradate	13.91	278.1883	APCI	Industrial
Biscayne Bay and canals	Marinobufagenin	13.42	400.2240	APCI	Natural product
Biscayne Bay and canals	memantine	12.90	179.1669	APCI	Pharmaceutical
Biscayne Bay and canals	Methcathinone	10.02	163.0995	APCI	Pharmaceutical
Biscayne Bay and canals	Myristyl sulfate	13.93	294.1842	APCI	Pesticide
Biscayne Bay and canals	N1-(9,10-Dihydrophenanthren-2-yl)acetamide	13.63	237.1130	APCI	Other
Biscayne Bay and canals	Naphthaleneacetamide	14.24	185.0839	APCI	Pesticide
Biscayne Bay and canals	Nordihydroguaiareticacid	11.95	302.1516	APCI	Natural product

Biscayne Bay and canals	Oxidized Latia luciferin	13.39	194.1668	APCI	Food additives, fragrance and dye
Biscayne Bay and canals	p-Octylacetophenone	13.74	232.1823	APCI	Industrial
Biscayne Bay and canals	Phthalic Acid, Bis-Propyl Ester	12.94	250.1211	APCI	Industrial
Biscayne Bay and canals	Prostaglandin F2α 1-11-lactone	14.36	318.2204	APCI	Pharmaceutical

These features with MS^2 library matches were further narrowed down to the top 5 candidates based on feature prioritization using the 5 tentatively identified chemicals unique to each source of water with highest peak areas to help identify characteristic compounds that can potentially be used as a representation of each water type (Table 3.7). Interestingly, this top 5 prioritized chemicals resulted in characteristic compounds found uniquely to each source of water as well as no overlapping compounds for ESI and APCI, expect for one of the compounds that were found in Biscayne Bay and in the Everglades. Information on each priority chemical detected, including classes and uses, peak area and ionization method are summarized in Table 3.7. The use of NTA characteristic features and unique composition makeup of each water body can potentially be used as a chemical fingerprint of each source for water source differentiation and tracking (Du et al., 2020).

Table 3.7 Top 5 candidates for each water source based on peak area.

Water Source	Top 5 candidate	Categories/Uses	Peak Area	Ionization method
Tap water	Benzoyl-y-tropeine	Drug/ Cocaine-related alkaloid	1468249	ESI
Tap water	(+)-brefeldin A	Fungal metabolite	917328	ESI
Tap water	Arenediol	Unknown	730137	ESI
Tap water	Tetrahydropteridine	Human metabolite	519870	ESI
Tap water	Fenuron	Herbicide	233130	ESI
Tap water	4-(1,4-Dioxaspiro[4.5]dec-8-yl)cyclohexanone	Unknown	1190792	APCI
Tap water	2-Hydroxyatrazine	Herbicide transformation/ Atrazine metabolite	685811	APCI
Tap water	Dodecylsuccinic Anhydride	Unknown	476738	APCI
Tap water	Arecoline	Nootropic drug	421017	APCI
Tap water	Phenacetin	Pharmaceutical/Analgesic	245123	APCI
Everglades National Park	4,5-Dioxo-4,5-dihydro-3-furancarbaldehyde	Flavorant and skin conditioner	414154	ESI
Everglades National Park	3-Hydroxycotinine(3HC)	Cotinine metabolite/Tobacco	391767	ESI
Everglades National Park	2-Ethylhexyl 4-(dimethylamino)benzoate	Sunscreen agent	390995	ESI
Everglades National Park	UNII:FL8S7F2JJQ	Hydroxy Fatty acid	214372	ESI
Everglades National Park	Methyl 2-chloro-1,3-oxazole-4-carboxylate	Unknown	201478	ESI
Everglades National Park	3-Hydroxy-4-(2-hydroxy-6-methyl-2-heptanyl)benzoic acid	Unknown	8674468	APCI
Everglades National Park	BJ0YZC8ZY8	Preservative and fragrance	8180365	APCI
Everglades National Park	Stearic acid	Fatty acid	4975763	APCI
Everglades National Park	2-(8-Hydroxy-4a,8-dimethyldecahydro-2-naphthalenyl)acrylic acid	Natural Product/medicinal plant Inula viscosa	4601919	APCI
Everglades National Park	2-[(2S,4aR,8aS)-2-Hydroxy-4a-methyl-8-methylenedecahydro-2-naphthalenyl]acrylic acid	Unknown	3226052	APCI

Biscayne Bay and canals	3-Cyclopropyl-1-methyl-1H-pyrazol-5-amine	Colorant	2917965	ESI
Biscayne Bay and canals	Eplerenone	Antihypertensive drug	2005029	ESI
Biscayne Bay and canals	Triethylenediamine	Adsorbant, adhesive	1621084	ESI
Biscayne Bay and canals	1-(1-Methyl-4-piperidinyl)piperazine	Unknown	205448	ESI
Biscayne Bay and canals	2,4-Dimethyl-1,3-diphosphete	Unknown	201299	ESI
Biscayne Bay and canals	Ethyl paraben	Preservative	29403013	APCI
Biscayne Bay and canals	4-Ethoxy ethylbenzoate	Industrial/Manufacturing	25287710	APCI
Biscayne Bay and canals	1-Stearoylglycerol	Fatty acid derivative	7309491	APCI
Biscayne Bay and canals	Nordihydroguaiareticacid	Antioxidant	4143443	APCI
Biscayne Bay and canals	4-Nonylphenol	Lubricating oil additives, resins, plasticizers	3498168	APCI

3.3.2 ENTACT inter-laboratory study

The initial analysis of the ENTACT samples were done blinded (i.e., information regarding what is present in these complex mixtures were not revealed until the experimental analysis was completed) to avoid any bias and to represent a "real-case" NTA scenario where the chemicals present in an actual environmental sample would not be known, therefore elucidating the limitations of the proposed NTA approach in the detection and identification of the compounds. An unblinded analysis of the ENTACT samples was performed to evaluate the limitations of the proposed NTA workflow in the detection and identification of the compounds present in the mixtures and to elucidate the differences between ESI and APCI methodologies.

The initial blinded analysis of the ENTACT samples revealed the detection of 1712 unique features by ESI and 1669 features by APCI at a confidence level of at least 3 (tentative candidate) according to the Schymanski scale (Schymanski et al., 2014a). It is shown that half of these features were at a confidence level of 2 (probable structure) (Schymanski et al., 2014a). This interpretation of the results can be seen in the Venn diagram in Figure 3.5. Among the features detected, only 491 were found to be in common to ESI and APCI at a confidence level of 3 and 276 found in common with a confidence level of 2, suggesting that APCI and ESI are complementary techniques, and a lot would be missed by choosing a single ionization technique.

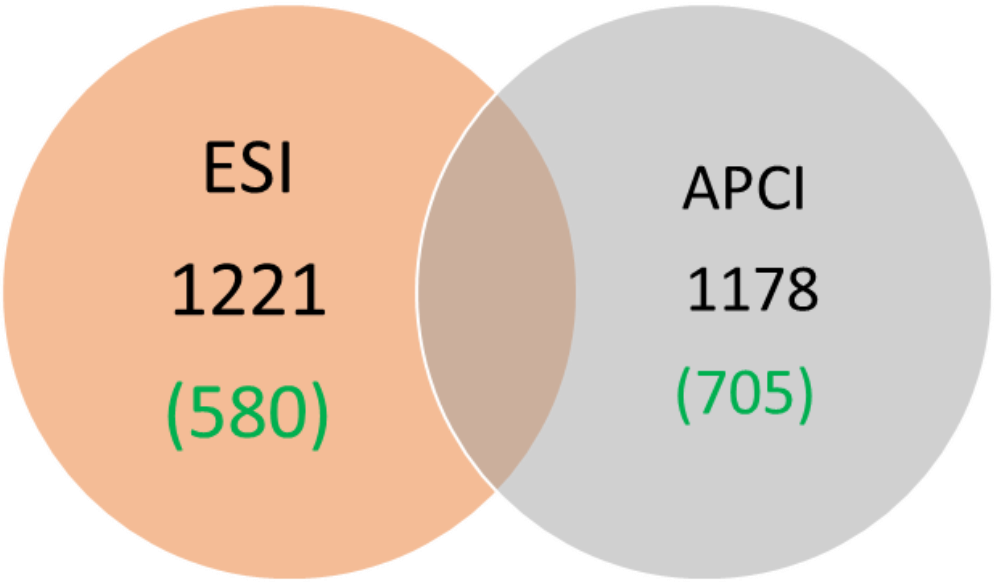


Figure 3.5 Blinded results of ENTACT samples. The number of features represented in black were obtained at a confidence level of 3 and green represents the number of features with a confidence level of 2.

Table 3.8 shows the results of the blinded ENTACT analysis after disclosure of the chemicals present in the different mixtures. A list of detected features was generated after post-processing by the software CD for both ESI and APCI methods. Results were divided into unattended observed features, which were provided directly by the software, and judged observed features, which consisted of additional manual data post-processing aimed to reduce noise and falsely identified compounds. The obtained results were consistent among the samples and methods, with 18-28% (% Hits) of the spiked chemical compounds detected in ESI and 16-28% of these detected in APCI at a confidence level of 3. The unattended data processing procedure resulted in a large amount of detected features, which included a lot of falsely identified compounds that were not spiked into the mixtures, but we demonstrated that with human intervention and additional post-processing steps, false positives can be reduced by almost up to 50% (Ng et al., 2020). Even after manual post

processing steps, the "judged" results still showed in some cases a slightly higher number of features detected in the ENTACT samples relative to the number of known chemicals present in those samples. Although more restrictive steps could have been taken to potentially reduce the number of false positives (e.g., reduce of number of databases, increase intensity thresholds, etc.), this could also lead to increasing number of false negatives (chemicals that are in the samples but are not detected).

Table 3.8 Blinded non-targeted analysis of EPA's ENTACT standard mixtures.

		unattended		judged			
ENTACT Mixture ID	Spiked Compounds	Observed features ESI	Observed features APCI	Filtered features ESI	Filtered features APCI	%Hits ESI	% Hits APCI
BF00173499	95	958	805	209	257	**28.4**	**26.3**
BF00173500	95	692	834	245	196	**17.9**	**22.1**
BF00173501	95	779	677	211	175	**22.1**	**21.1**
BF00173502	95	829	813	240	146	**23.2**	**15.8**
BF00173503	185	1027	1432	200	191	**20.0**	**18.9**
BF00173504	185	1125	1519	268	221	**23.2**	**23.2**
BF00173505	365	1532	2019	241	325	**20.8**	**27.7**
BF00173506	365	1451	2455	247	310	**25.2**	**23.0**
BF00173507	95	957	1000	134	117	**20.0**	**16.8**
BF00173508	365	1513	1955	243	314	**18.4**	**16.4**
Total	**1940**	**10863**	**13509**	**2238**	**2252**	**21.7**	**21.6**

To better understand and evaluate the impact of database searched has on NTA, the data was reprocessed unblinded (after it was revealed what was spiked into each sample mixture) and the reprocessing of the raw data files was done by restricting the searched

database to the EPA ToxCast database from which the chemical substances were taken to make the ENTACT sample mixtures. The restricting of the number of searched databases resulted in an increased ability to detect 36-54% of the spiked chemical compounds in ESI and 40-63% in APCI. The unblinded analysis showed that the majority of the compounds (n=788) were detected by either ESI or APCI, while 300 were detected only by APCI and 123 only by ESI (shown below in Figure 3.6). In the unblinded analysis using both ionization techniques, 1211 compounds out of the 1940 present compounds (62%) were detected. We believe that compounds that were not detected in our NTA workflow are likely due to a combination of low concentrations (after dilution), poor ionization characteristics (influenced by mobile phase additives, source, etc.), and physical chemical characteristics, e.g., more amenable to gas-chromatography-mass spectrometry (GC-MS).

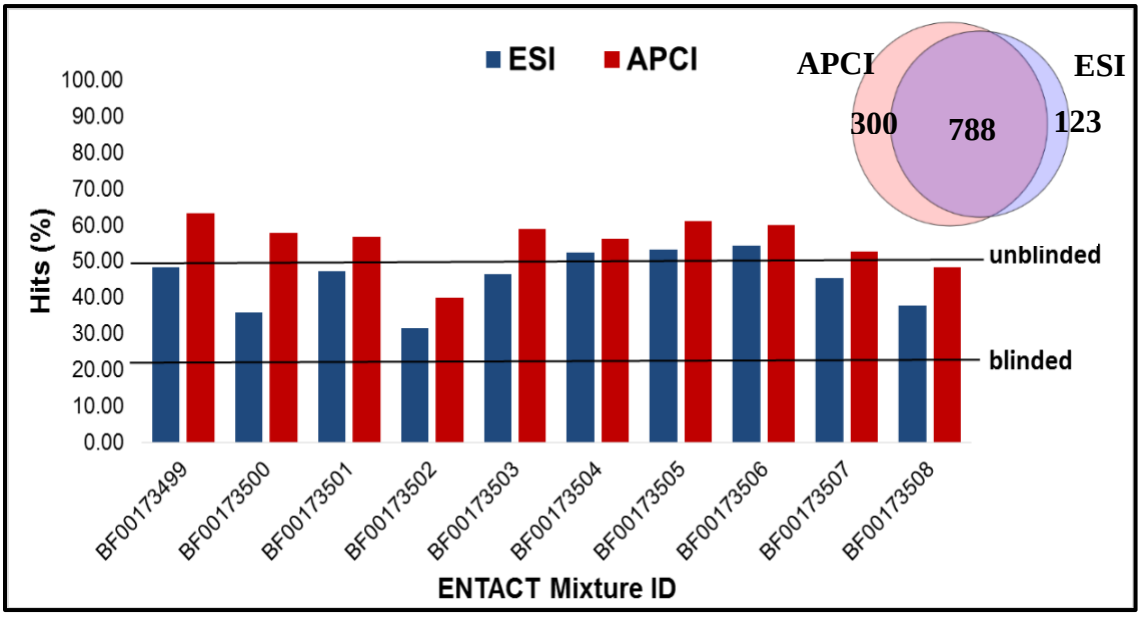


Figure 3.6 Blinded and unblinded non-targeted analysis of EPA's ENTACT standard mixtures. % Hits being the % of spiked compounds in the sample being detected. Lines represent the average result for blinded and unblinded analysis.

The results for this study were different from that of (Singh et al., 2020) in which they observed better performance for ESI than APCI. Their study found that ESI detected 78-88% of the spiked chemical compounds, whereas APCI detected 60-78%. There are multiple factors that impact performance and can influence the results such as the different chromatography parameters (mobile phase solvents, modifiers and gradient used), instrumental and ionization source parameters and most importantly, the data analysis process.

Although ESI is the most commonly used ionization source for NTA, the use of both ESI and APCI for NTA would expand the chemical space coverage and should be considered, especially for tough to ionize chemical compounds. Although the EPA ToxCast database was one of many other databases (a total of 8 were used) searched during the blinded study, as expected by restricting the database being searched has improved the method performance for the identification of the chemicals present in the complex mixtures. This can be attributed to the algorithm responsible for the decision and assignment of chemical annotation and/or compound identification to specific features. However, when performing NTA on environmental samples, especially when no prior information is known on potential sources of pollutions (i.e. it is not known which organic contaminants can potentially be present), the restriction on the number of databases searched to a single or smaller database would introduce biases and a lot may be missed.

The use of multiple large databases provides a wider coverage of chemicals for detection, nevertheless, it may also further complicate the already convoluted NTA post-processing step, increasing the possibility of wrong assignment to feature identification (e.g., increase of false positives), thus reducing method accuracy related to compound identification in the sample.

3.3.3 Understanding the chemical space covered by ESI and APCI

Overall, positive mode ionization detected more features than negative mode ionization for both ESI and APCI. While ESI detected approximately 3 times more features in positive compared to negative mode in the environmental samples, the number of features detected in positive and negative mode by APCI were similar (3079 vs 3013 features, respectively), as seen in Fig S2. This increased number of detections in APCI negative is expected since APCI has shown to increase chemical space coverage by aiding the ionization of compounds in negative mode (Singh et al., 2020). These positive and negative features detected by APCI and ESI in the studied water sources were summarized in Figure 3.2.

A Kendrick mass defect (KMD) plot offers another way to visualize data by adding a second dimension to the mass spectra and simplifying the identification of ions (Fouquet, 2019; Kendrick, 1963). A KMD plot is a graphical representation of the difference between the nominal mass and exact Kendrick mass against the Kendrick nominal mass (KNM). Kendrick mass is defined as the IUPAC mass x 14.00000/14.01565. The use of a KMD plot for NTA data interpretation can help reduce the massive spectral data usually obtained by restricting compounds within the same homologous series to a fixed 14 mass unit

intervals (CH_2) as they tend to share the same KMD (Jobst et al., 2013; Ubukata et al., 2015). This leads to a much simpler plot in which distinctive patterns for homologous organic compounds can be observed (Sleno, 2012). Different KMD plots were obtained for ESI and APCI analysis of the same environmental water samples as seen in Figure 3.7, demonstrating the visual difference between the ionization techniques, with APCI showing higher ionization efficiency and having more features detected than ESI (as also shown in Figure 3.2). Within each KMD plot, there are regions of common overlap between the sources of water from the Everglades National Park, Biscayne Bay and its related canals and tap water, but more importantly are the regions of non-overlap, indicating unique features for each different water source. One of the most evident differences observed in Figure 3.7 is that features with larger molecular weight (KNM> 300) are more frequently detected using APCI for all water types in comparison to ESI.

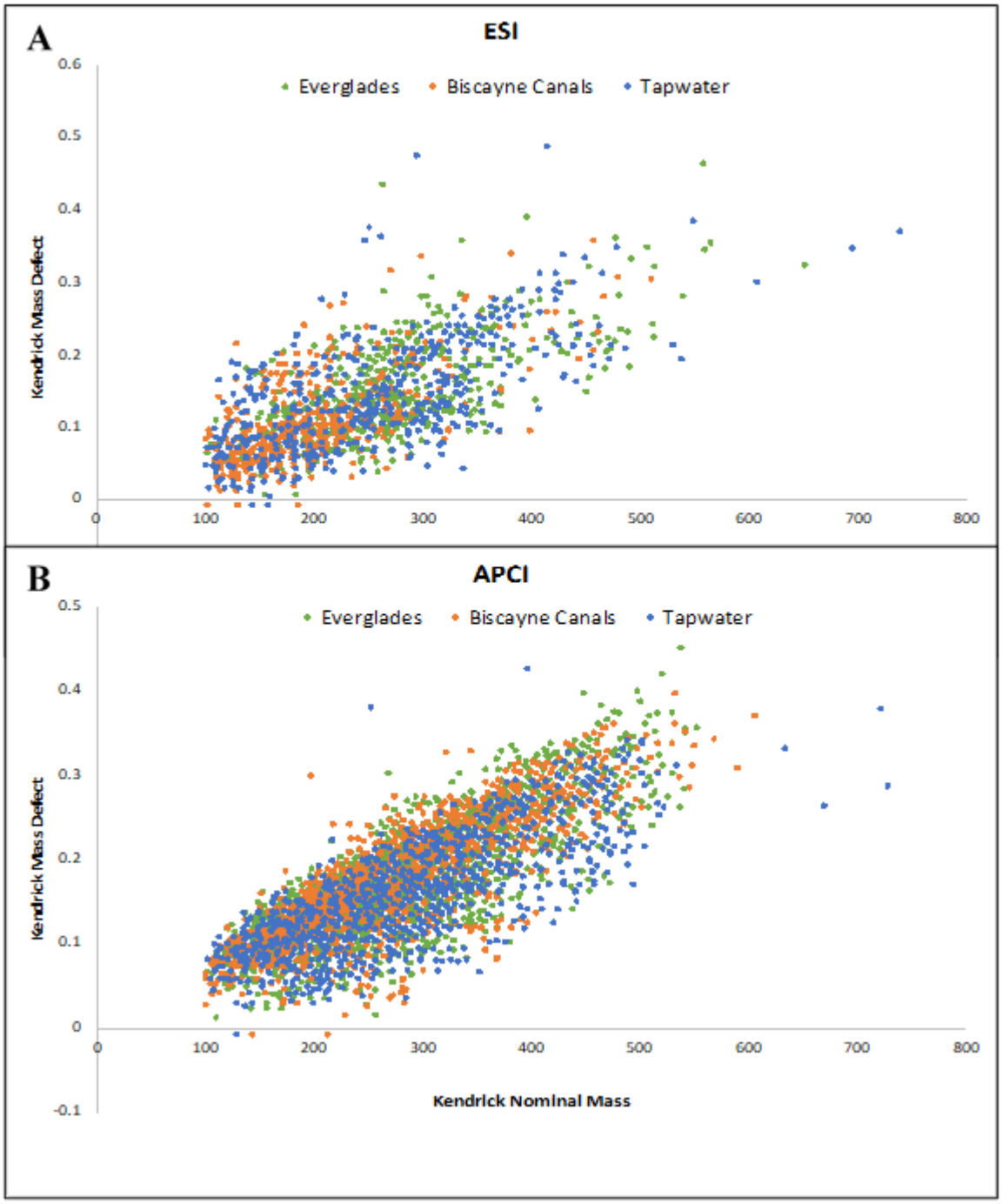


Figure 3.7 Kendrick mass defect plot comparison of various water sources for (A) ESI and (B) APCI.

To better visualize the chemical space with respect to KMD, some classes of compounds belonging to the same homologous series were taken from the EPA's DSSTox database and plotted. As can be seen in Figure 4, chemical compounds belonging to the same homologous series tend to have very similar KMD and important distinct patterns can be seen. These closely related chemical compounds tend to cluster in a linear pattern. This distinctive pattern is observed for compounds with varying chain lengths such as polyethylene glycol (PEG)/polypropylene glycol (PPG), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides, per- and polyfluoroalkyl substances (PFAS), polysiloxane, bisphenol and phthalates, surfactants, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), with polymeric chains of homologous series often having the same KMD and appearing horizontally on a KMD plot (Baduel et al., 2017; Ishitsuka et al., 2020; Jobst et al., 2013). Another distinctive feature of KMD is that halogenated compounds tend to have a negative mass defect. This negative mass defect can help shift halogenated compounds into a much less densely populated region of the complex mass spectra for easier identification. Although, we can observe that not always chemicals containing halogens will fall within this region, having in some cases a positive mass defect as seen in Figure 3.8.

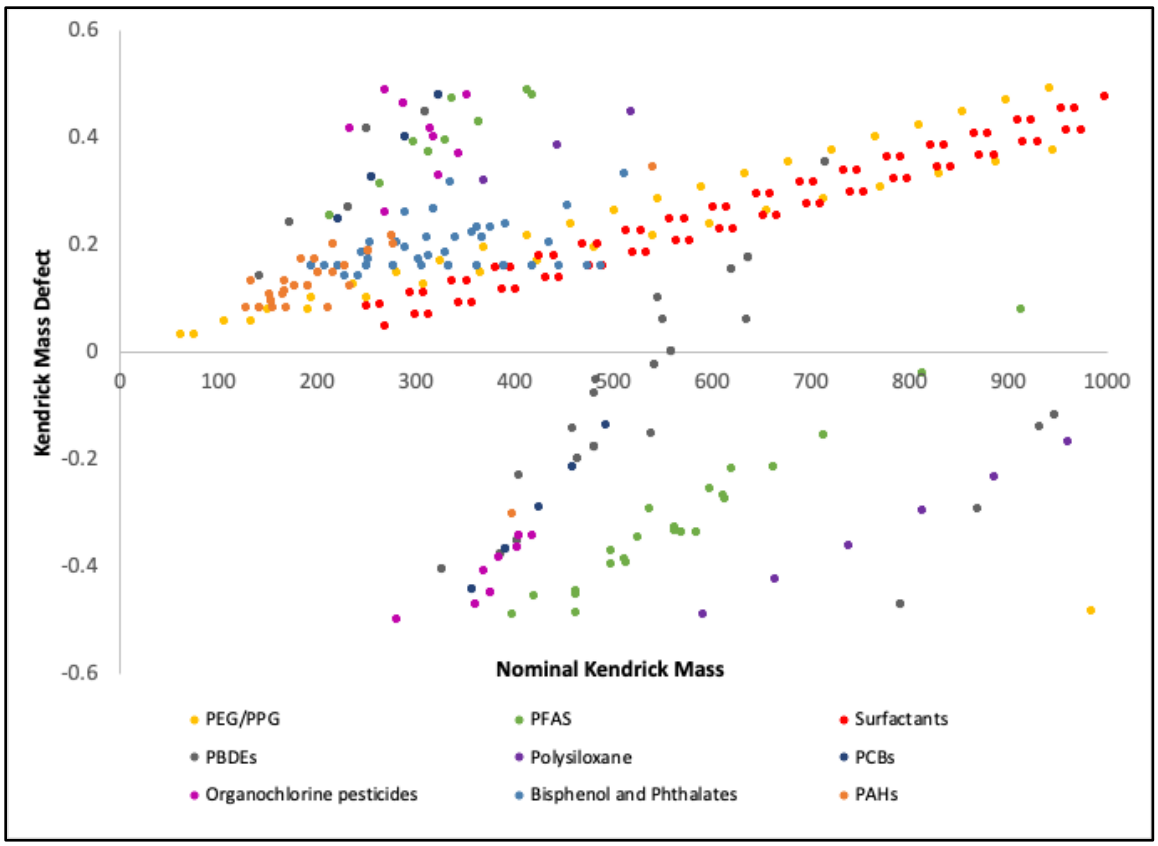


Figure 3.8 Theoretical Kendrick mass defect plot of some contaminants of concern taken from the EPA's DSSTox library.

However, an issue with KMD plots is that it can be difficult to identify these homologous series in complex mixtures with many features. An additional graphical analysis method that is often used in conjunction with KMD plots is the Van Krevelen diagram. A Van Krevelen diagram is a plot of the atomic ratio of hydrogen to carbon (H/C) against the atomic ratio of oxygen to carbon (O/C) of a compound (Wu et al., 2004). This further separates compounds based on their degree of saturation (H/C ratio) and by classes containing oxygen (O/C ratio). A Van Krevelen diagram of the previously mentioned chemical compounds taken from the EPA's DSSTox database were plotted as shown in Figure 3.9 and individual plots for each chemical class plotted in Figure 3.10.

Due to the chemical formula of PAHs containing mostly C and H and no O, they distinctively can be found along the y-axis of H/C. Other classes of compounds that shifts away from the densely populated region of the plot are PFAS, PCBs and PBDEs. This is due to PFAS, PCBs and PBDEs having a unique chemical formula in which the majority of the H are replaced with fluorine, chlorine or bromine atoms respectively, shifting them lower down on the van Krevelen diagram. Surfactants and PEG/PPG tend to be made up of long chains consisting of C and H, with surfactants generally having a hydrophilic head containing oxygen and PEG/PPG having long repeat chains that are linked together by an oxygen. These distinctive chemical makeup of surfactants and PEG/PPG shifts them into a high region of the van Krevelen diagram away from the clutter of the densely populated plot. These uniquely populated regions of the van Krevelen diagram by these classes of compounds are highlighted in Figure 3.9.

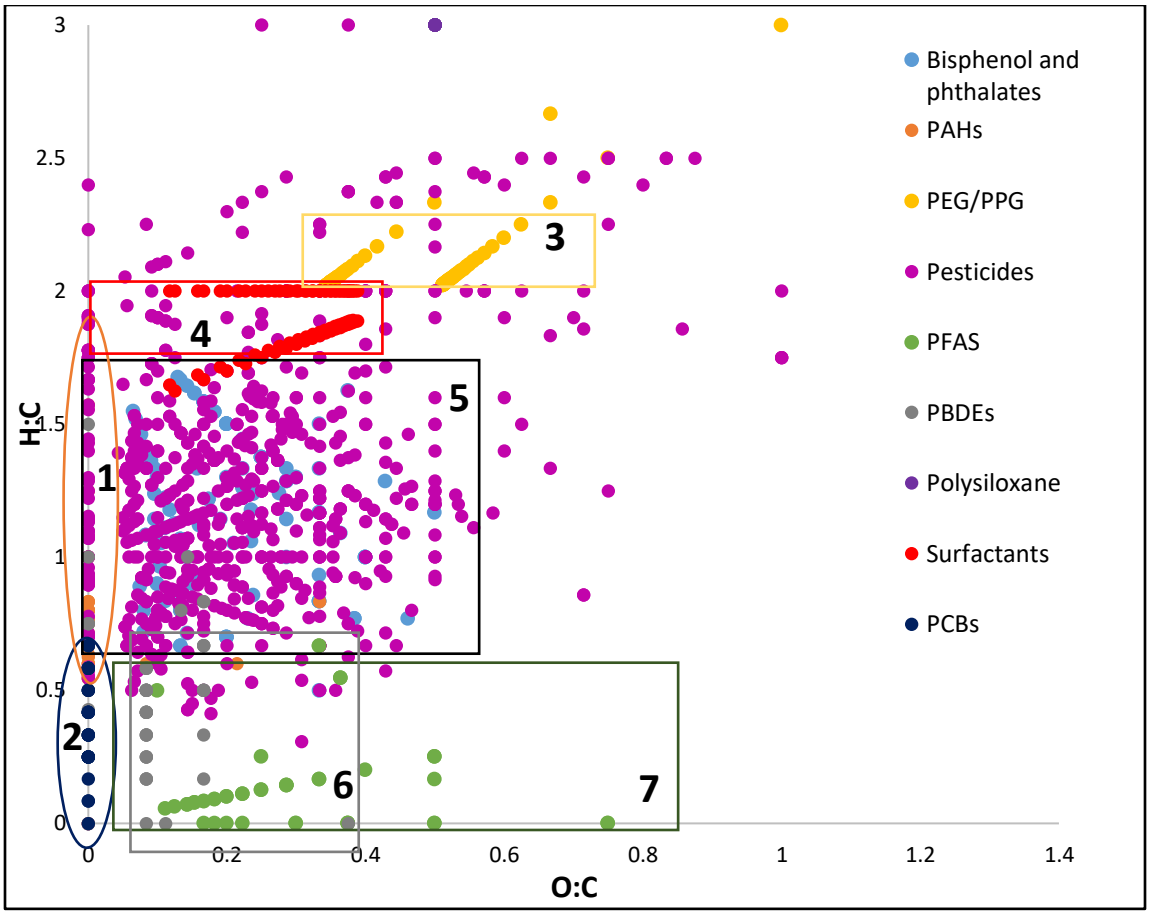
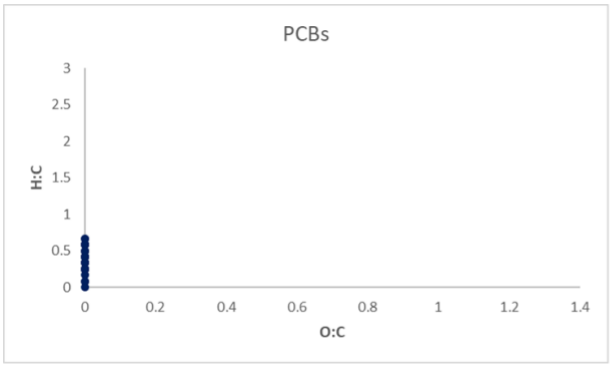
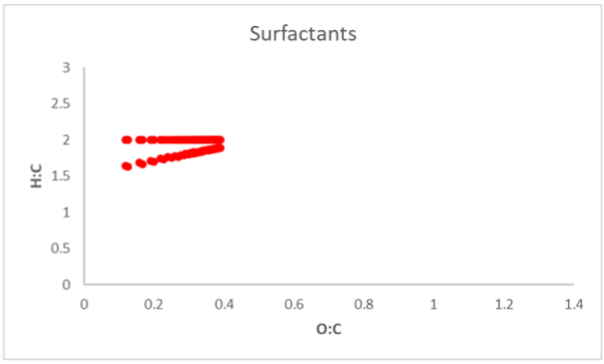


Figure 3.9 Theoretical Van Krevelen diagram of some contaminants of concern taken from the EPA's DSSTox library. Defined boxes as 1. PAH, 2. PCBs, 3. PEG/PPG, 4. Surfactants, 5. Pesticides, bisphenols and phthalates, 6. PBDEs and 7. PFAS.


PCBs
H:C
O:C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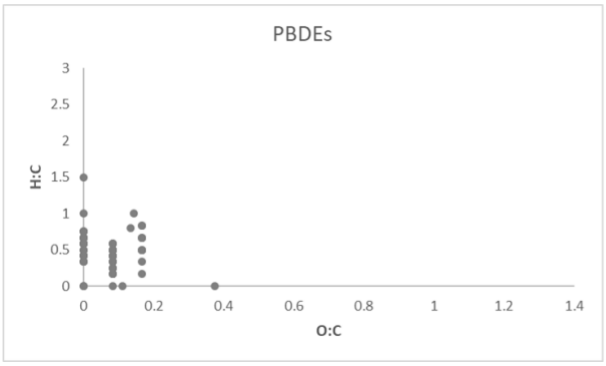
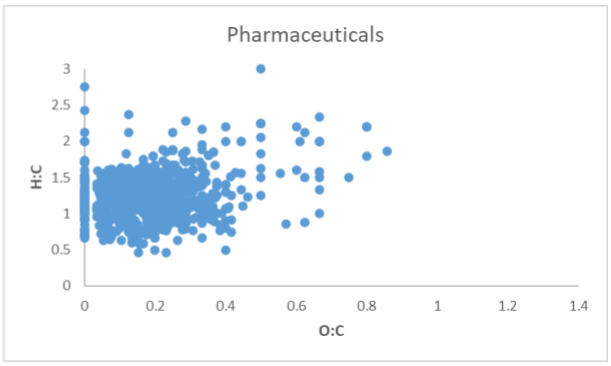
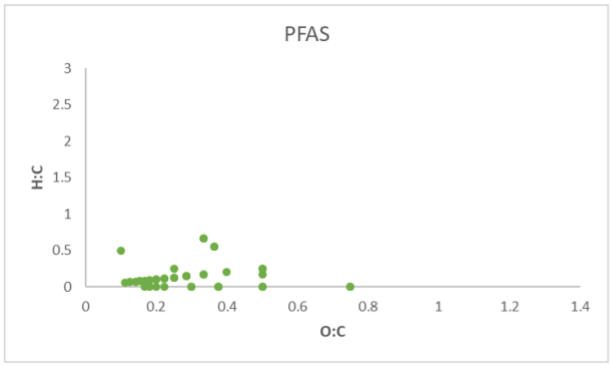
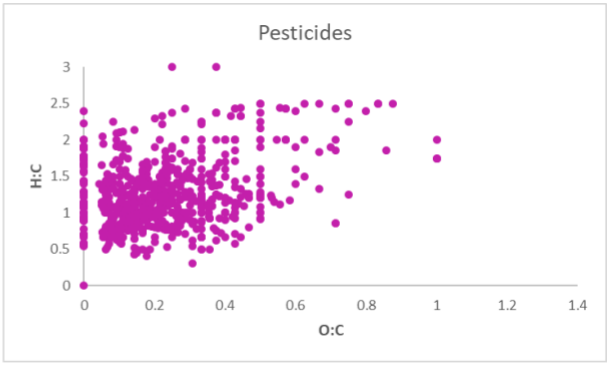
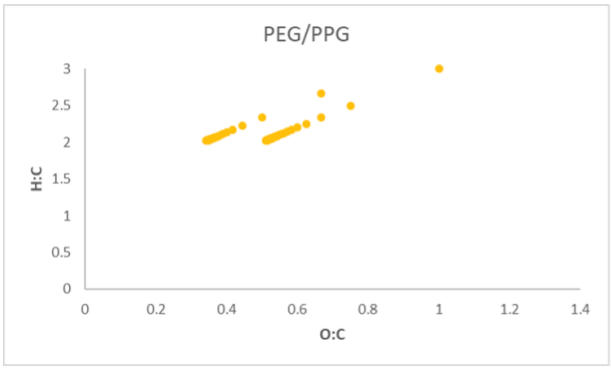
H:C
O:C

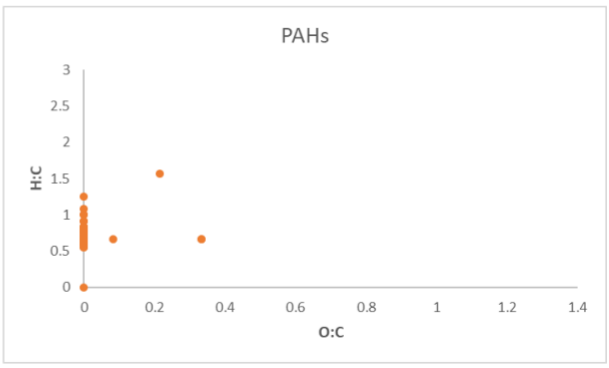

PBDEs
H:C
O:C


Pharmaceuticals
H:C
O:C


PFAS
H:C
O:C


Pesticides
H:C
O:C


PEG/PPG
H:C
O:C


PAHs
H:C
O:C

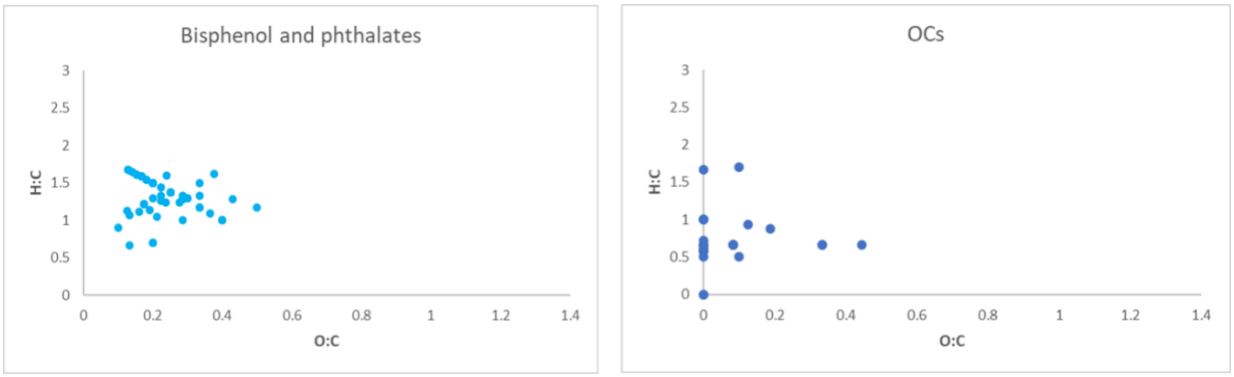


Figure 3.10 Van Krevelen diagram plots of each class of contaminants of concern taken from the EPA's DSSTox library.

Applying this concept of different classes of compounds tending to occupy different regions of the van Krevelen diagram to the ESI and APCI results of environmental surface samples (Figure 3.11), it can be seen that for both ESI and APCI, the regions of PAHs, surfactants, pesticides, bisphenols and phthalates are most densely populated, with the higher H/C regions for PBDEs and PCBs being more densely populated for APCI than ESI. It's important to highlight that PAHs, PBDEs and PCBs are usually detected by GC-MS, although the use of LC-MS with stronger ionization sources such as atmospheric pressure photoionization (APPI) has been already demonstrated that these chemical classes can be analyzed by LC-MS (Huba et al., 2016; Moukas et al., 2014). This concept which was previously used for dissolved organic matter (DOM) to categorize regions that can be associated with proteins, lipids, carbohydrates, lignins and tannins (Maizel and Remucal, 2017; Verkh et al., 2018), is being here proposed to address regions in the van Krevelen diagram related to anthropogenic chemicals such as legacy and emerging organic contaminants of concern, showing a better visualization and representation of the chemical space within the samples, which could be potentially further improved by encompassing a

much larger list of chemical compounds to reveal new anthropogenic spaces. The proposed regions defined in the theoretical Van Krevelen diagram could be used not only for LC-MS, but also for GC-MS applications.

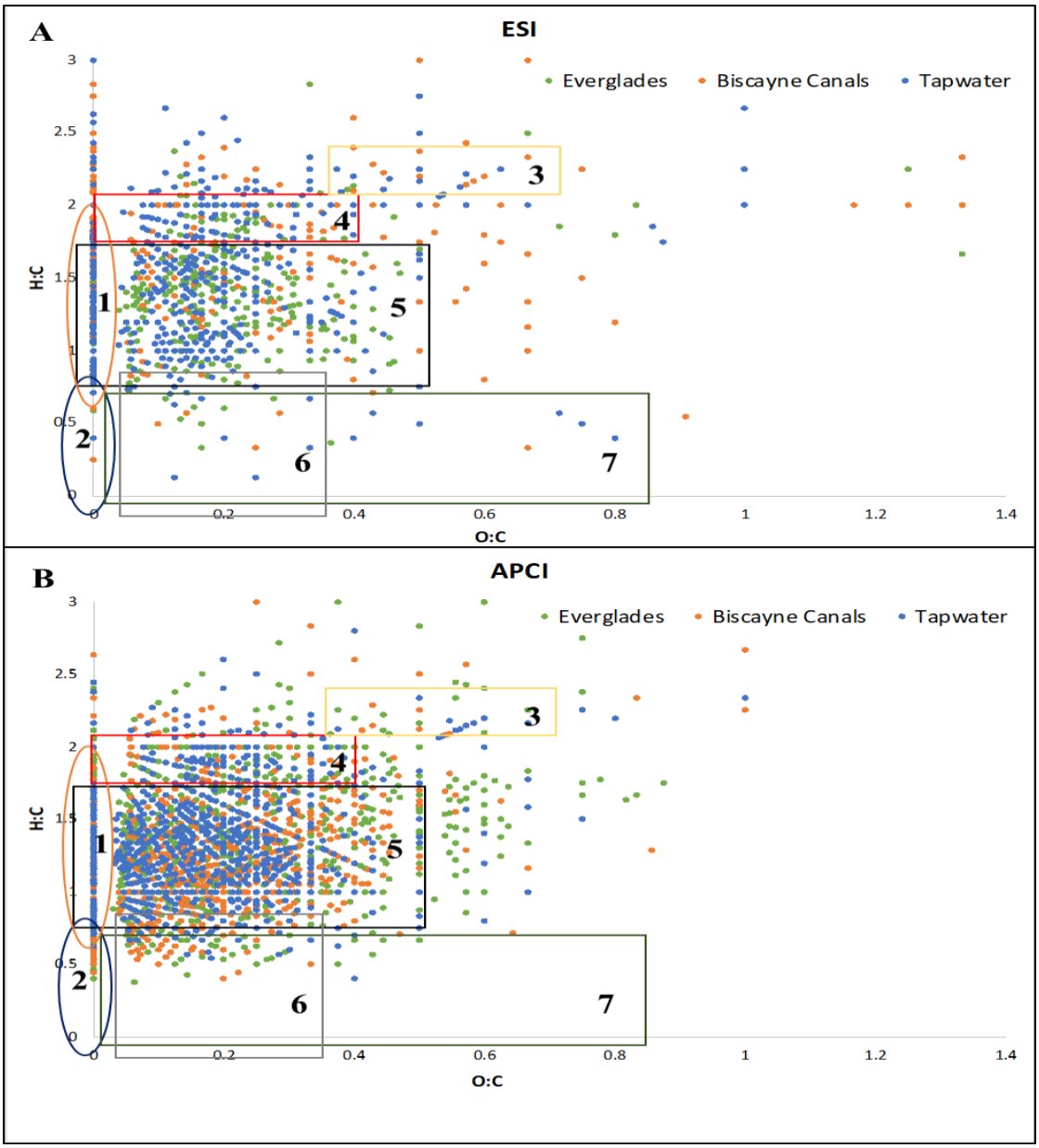


Figure 3.11 Van Krevelen diagram comparison of various water sources for (A) ESI and (B) APCI. Defined boxes as 1. PAH, 2. PCBs, 3. PEG/PPG, 4. Surfactants, 5. Pesticides, bisphenols and phthalates, 6. PBDEs and 7. PFAS.

3.4 CONCLUSIONS

In this study it was found that APCI is complementary technique to ESI for the purpose of NTA and that there are chemical compounds that would be overlooked by just choosing a single ionization technique. Database selection is extremely important for NTA as selecting too many or too large databases can complicate the complex structure elucidation process and lead to increased false identification of chemical compounds, while selecting a single or smaller database can lead to bias and overlook a lot of compounds of interest that may actually be present. The use of additional graphical methods, such as Kendrick mass defect plots and van Krevelen diagrams, can help in understanding and identification of the chemical space covered by ESI and APCI for the purpose of NTA. The different water bodies studied have shown reproducible features (i.e., present in all samples) and distinct chemical composition makeup that are characteristic to each source. The NTA workflow could be used not only for prioritization of chemicals of environmental concern, but also for the identification of specific tracers for source tracking and differentiation.

3.5 ACKNOWLEDGMENTS

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CHAPTER 4 A SIMPLE POLYDIMETHYLSILOXANE (PDMS) SPONGE FOR THE REMOVAL OF ORGANIC CONTAMINANTS FROM RUNOFF WATERS

4.1 INTRODUCTION

Water is one of the most essential natural resources and fundamental to sustaining life on Earth. In addition to drinking, water plays an important role in industrial activities, agriculture and the rearing of livestock; all of which are vital to the economy (Sharma and Bhattacharya, 2017; Tyagi et al., 2013). Due to the growing population and accompanying increase in industrialization and urbanization, large amounts of pollution enter water in the environment via industrial discharge, residential and commercial wastewater, floating debris and polluted stormwater runoff. These anthropogenic influences are major sources of organic contaminants and can enter environmental waters as hazardous chemicals, pharmaceuticals, personal care products, insecticides and/or pesticides and contribute to a decline in water quality, hindering its uses (Sharma and Bhattacharya, 2017; Simeonov et al., 2003). Organic contaminants can have adverse health effects such as disruption of the endocrine system and carcinogenic effects, therefore their removal from the aquatic environment is important to avoid their potential perilous health effects (Sirés and Brillas, 2012; Snyder et al., 2003).

Stormwater runoff has been a major source of contaminants in the urban environment (Müller et al., 2020; Werbowski et al., 2021); its physical, chemical and microbial composition makeup is based on the surfaces that it came into contact such as roads and roofing (Eriksson et al., 2007) but also green spaces that are heavily used for recreation and transit. This runoff have been found to contain bacterial pathogens (Ahmed et al., 2019; Ahmed et al., 2018), tire particles (Tian et al., 2021b; Werbowski et al., 2021), pesticides (Hou et al., 2019), suspended solids and polycyclic aromatic hydrocarbons (PAHs) (Aryal et al., 2010; Brown and Peake, 2006), and endocrine disrupting chemicals

(EDCs), pharmaceuticals and personal care products (Boyd et al., 2004). This results in waterbodies having lower water quality and can results in adverse effects to aquatic organisms. One such example is the acute mortality in adult spawning coho salmon when they encounter freshwater containing stormwater runoff (McIntyre et al., 2018; Tian et al., 2021b). Therefore there is a need for preventative measures which can help alleviate the amount of contaminants from stormwater runoff entering into the environment.

One possible solution can be polydimethylsiloxane (PDMS) sponge hybrid composites that can act both as a physical filter towards debris, an adsorbent for chemical contaminants and a scaffold that deactivates bacteria. PDMS is a commercially available, low-cost silicone elastomer that is chemically inert, thermally stable, malleable as well as biocompatible and nontoxic (Jo et al., 2000; Mata et al., 2005). These properties have made PDMS widely used in medical applications, passive environmental sampling (DiFilippo and Eganhouse, 2010; Villar et al., 2018) and for the removal of oil from water (Choi et al., 2011; Park et al., 2009; Wang and Lin, 2013). Due to its polymer network structure, it is highly permeable compared to other materials. This porous property facilitates the diffusion of small molecules into the polymer (Toepke and Beebe, 2006) and have been shown to extract organic compounds from aqueous solution (Theodoridis et al., 2004). Due to its porosity and hydrophobicity, there is potential for compounds to adsorb to the PDMS matrix (Nianzhen et al., 2009; Villar et al., 2018) and this adsorption of small molecules by PDMS poses an issue in the field of microfluidic devices as it retains small molecules, making it unavailable for detection and is considered a drawback (Mukhopadhyay, 2007; Toepke and Beebe, 2006). However this same property of PDMS is valued and utilized in the field of passive environmental sampling of hydrophobic compounds (Bragg et al.,

2006; DiFilippo and Eganhouse, 2010) and in a variety of analytical techniques such as solid phase microextraction (SPME) (Mayer et al., 2000; Tuduri et al., 2001), fabric phase sorptive extraction (Kabir and Furton, 2016; Sun et al., 2019), PDMS disk (Heltsley et al., 2005), silicon o-rings (Stibany et al., 2020; Stibany et al., 2017) and stir bar sorptive extraction (SBSE) (Baltussen et al., 1999; David and Sandra, 2007) to pre-concentrate organic compounds through partitioning into the PDMS (Jahnke and Mayer, 2010). Previous studies have shown that the oleophilic and hydrophobic nature of PDMS sponges has made it ideal for the selective absorption of oil from water and potential to absorb a variety of organics that occur naturally in crude oil, mainly benzene, toluene, ethylbenzene and xylene (commonly known as BTEX) (Choi et al., 2011; Park et al., 2009). The work described by Choi et al. uses a soluble support material that creates pores in the PDMS whose sizes are controllable during the manufacturing process.

In this manuscript, we describe the manufacturing and testing of a simple and inexpensive PDMS sponge composite that can be made from low cost, commercially available reagents and demonstrate their potential application in the removal process of organic contaminants in polluted waters. Such sponge was then functionalized to assess if other adjuvants will modify its properties towards increased adsorption (addition of carbon) or inactivation of microorganisms (addition of micron-size copper). We initially evaluated the sorption of various compounds to lab made and customized PDMS sponges in order to determine the performance. To accomplish this, compounds spanning a range of Log K_{ow} 0.16 to 6.26 were used in the experiment. At latter stages, addition of activated carbon was used to increase the adsorption properties and copper was used as a biocide towards *E. coli*.

4.2 MATERIALS AND METHODS

4.2.1 Chemicals and reagents

Standards and reagents were purchased from commercial vendors. Water, Acetonitrile (ACN), Methanol (MeOH), and formic acid (FA) were all Optima LC/MS grade purchased from Fisher Scientific (Fair Lawn, NJ, USA). The following were used in this study: Sylgard 184 silicone elastomer kit (Dow Corning), commercially available store brand 1x1x1 cm sugar cubes, caffeine (>98.5% purity, Sigma-Aldrich), lincomycin (>90%, Sigma), sulfamethoxazole (>99%, Sigma), trimethoprim (>98%, Sigma), norcocaine (>99%, Cerilliant), carbamazepine (>99%, Sigma-Aldrich), (+)-cis-diltiazem hydrochloride (>99%, Sigma), atrazine (98%, Chem Service), diphenhydramine hydrochloride (>98%, Sigma), fluoxetine hydrochloride (100%, Sigma), sertraline hydrochloride (>99%, Sigma), clotrimazole (>98%, Sigma), atrazine D5 (97%, Dr. Ehrenstorfer GmbH), copper metal purified (electrolytic powder, Fisher Scientific, Fair Lawn, NJ, USA). The compounds were selected to cover a wide range of polarity and K_{ow} that can be detected by electrospray ionization (ESI) in positive mode. Sample preparation was done as outlined by Ng et al. The chemicals and their respective molecular formula, monoisotopic mass, octanol/water partition coefficient (log K_{ow}) and the monitored ions in ESI are listed in Table 4.1.

Table 4.1 List of compounds and their respective log K_{ow}, molecular formula, monoisotopic mass and monitored ions. Adapted from Ng et al., 2020.

Compound	Log K_{ow}	Molecular formula	Monoisotopic mass	Monitored ions
Caffeine	0.16	$C_8H_{10}N_4O_2$	194.0804	195.0877
Lincomycin	0.29	$C_{18}H_{34}N_2O_6S$	406.2137	407.2210
Sulfamethoxazole	0.48	$C_{10}H_{11}N_3O_3S$	253.0521	254.0594
Trimethoprim	0.73	$C_{14}H_{18}N_4O_3$	290.1379	291.1452
Norcocaine	1.96	$C_{16}H_{19}NO_4$	289.1314	290.1387
Carbamazepine	2.25	$C_{15}H_{12}N_2O$	236.0950	237.1022
Diltiazem	2.79	$C_{22}H_{26}N_2O_4S$	414.1613	415.1686
Atrazine	2.82	$C_8H_{14}ClN_5$	215.0938	216.1010
Diphenhydramine	3.11	$C_{17}H_{21}NO$	255.1623	256.1696
Fluoxetine	4.65	$C_{17}H_{18}F_3NO$	309.1341	310.1413
Sertraline	5.29	$C_{17}H_{17}Cl_2N$	305.0738	306.0811
Clotrimazole	6.26	$C_{22}H_{17}ClN_2$	344.1080	345.1153

4.2.2 Synthesis of PDMS Sponges

The preparation of PDMS sponges was adapted from previously published work by Choi et al. and according to the manufacturer's specification. Curing agent was added to the PDMS base in a ratio of 10:1 base to curing agent by volume, and mixed thoroughly. This mixture was transferred into a watch glass and 1x1x1 cm^3 sugar cubes were placed in the center of the mixture. This sugar cube template can easily be dissolved away by just placing in water and does not interact with the polymer. This setup was then placed into a vacuum chamber and left to degas for four (4) hours. During this degassing process, the PDMS/curing agent mixture infiltrates the sugar cube template via capillary action. After 4 hours, the PDMS/curing agent mixture has completely infiltrated the sugar cube template

and the watch glass was placed into an oven and heated at 120°C for 12 minutes. This thermal heating process completes the polymerization of the PDMS with the curing agent. The sugar cube template was then dissolved away by placing in a water bath at 70°C, leaving behind a microporous PDMS sponge. This process was repeated for the making of the charcoal and copper sponges. For the charcoal and copper sponges, charcoal was added to the PDMS base at a 1:10 ratio (w/w) and copper was added at a 1:5 ratio (w/w), and mixed thoroughly prior to the addition of the curing agent. Then the sponge manufacturing process repeated as described above. This simple synthesis process and low cost of materials can be easily scaled up to larger templates. In addition, the ease at which sugar can be molded into different shapes, sizes, easily be removed and the ability to adjust the pore size of the PDMS sponge meshwork based on sugar particle size makes it a very versatile template choice. Another feature that makes sugar an ideal template choice is that sponges of different pore sizes can be easily manufactured by using different sugar particle sizes such as black sugar (1500-1800 µm), sanding sugar (1000-1100 µm) and granulated sugar (400-500 µm) or a combination of them, which can improve the absorption capacity (Choi et al., 2011). However, the purpose of this study was to show proof of concept and not the optimization of performance, therefore cheap, readily available premade and uniform 1x1x1 sugar cubes were chosen as the template choice.

4.2.3 Characterization of Copper Sponges

The surface morphology of the copper sponge was characterized by field emission scanning electron microscopy (FESEM) (JSM-6330F, JEOL, Japan). Backscattered electron images were taken of the copper sponges to determine the surface composition and whether the copper particles are available on the surface to perform contact killing of bacteria.

4.2.4 High Resolution Mass Spectrometry Analysis

For the experiment, the prepared 200 ng/mL mixture was diluted with LC-MS water to a final concentration of 1ng/mL in 300 mL and a single 1x1x1 cm^3 PDMS sponge was added. At the following time intervals, a sample (10.5 mL) was taken out for analysis: 0, 0.5, 1, 2, 4, 8, 12 and 24 hours respectively. The analysis was done using a method previously developed and published by Ng et al., and summarized in Table 4.2 (gradient for the online-solid phase extraction pre-concentration step) and Table 4.3 (gradient for the elution and separation by the analytical column) of the supporting information. Analysis was carried out using an electrospray ionization source (ESI) in positive mode ionization with full scan at a resolution of 140,000, using a Q Exactive Orbitrap (Thermo Scientific, USA) by online-solid phase extraction (SPE) using a Hypersil GOLD aQ (20 x 2.1 mm, 12 µm, Thermo Scientific, USA) for pre-concentration and a Hypersil GOLD aQ C18 polar endcapped (100 x 2.1, 1.9µm, Thermo Scientific, USA) as analytical column for separation of the compounds of interest.

Table 4.2 Gradient of pump 2 for the online SPE pre-concentration step.

Pump 2 – Online SPE						
	Time	A%	B%	C%	D%	µL/min
0	0.00	98.0	0.0	2.0	0.0	200.0
1	0.10	98.0	0.0	2.0	0.0	2500.0
2	4.20	98.0	0.0	2.0	0.0	2500.0
3	4.50	0.0	0.0	100.0	0.0	1000.0
4	6.50	0.0	0.0	100.0	0.0	1000.0
5	7.00	10.0	90.0	0.0	0.0	1000.0
6	8.00	10.0	90.0	0.0	0.0	1000.0
7	8.90	98.0	0.0	2.0	0.0	1000.0
8	15.00	98.0	0.0	2.0	0.0	1000.0

A: water, B: methanol, C: acetonitrile, D: 0.1% formic acid

Table 4.3 Gradient of pump 1 for the elution and separation step by the analytical column.

Pump 1 – Analytical column						
	Time	A%	B%	C%	D%	µL/min
0	0.00	0.0	0.0	2.0	98.0	250.0
1	4.20	0.0	0.0	2.0	98.0	250.0
2	10.00	0.0	0.0	60.0	40.0	250.0
3	12.00	0.0	0.0	100.0	0.0	250.0
4	15.00	0.0	0.0	100.0	0.0	250.0

A: water, B: methanol, C: acetonitrile, D: 0.1% formic acid

4.2.5 E. coli

The copper sponge evaluation was done based on its ability to inactivate *E. coli* via contact killing. This was done using ColiPlate™ kits obtained from Bluewater Biosciences (Toronto, ON, Canada) for the quantitative determination of *E. coli* bacteria. This test is designed to meet regulatory guidelines for surface water, recreational water, processing water and wastewater. The ColiPlate™ kits were used as per manufacture instruction, in

which 100 mL of water sample was dispensed into the microplate and then incubated at 35 °C for 24 hours. The presence of blue and fluorescent wells are indicative of *E. coli.* The test quantifies density of target bacteria, *E. coli*, ranging from 5 to 5,000 colony forming-units (cfu) per 100 mL sample, without dilutions (Ng et al., 2021). ColiPlate tests were performed on water samples collected in the Miami area (Coral Gables waterway) in 2021. Water samples from Coral Gables waterway were chosen for the test of the copper sponge in reducing *E. coli* because it was previously reported to be a contaminated water system with the presence of *E. coli* confirmed (Smith et al., 2021). The collected samples were transported on ice and processed within 6 hours of collection. The samples on arrival were poured into separate beakers; without copper sponge (control) and with a single 1x1x1 cm^3 copper sponge added and left at room temperature for 4 hours prior to the quantification of *E. coli* using the ColiPlate™ kits.

4.3 RESULTS AND DISCUSSION

After placing a single 1x1x1 cm^3 PDMS sponge into a solution containing 1 ng/mL of each compound, the sponge was left over a 24 hour period and samples were taken out at various time intervals for analysis. The adsorption of each compound over 24 hours was plotted and shown in Figure 1. The PDMS sponge showed greater affinity towards compounds with larger Log K_{ow}. As observed in Figure 4.1, adsorption began from norcocaine (Log K_{ow} 1.96) and gradually increased as you go to compounds of higher Log K_{ow} with clotrimazole (Log K_{ow} 6.26) having the greatest adsorption. Caffeine (Log K_{ow} 0.16) and lincomycin (Log K_{ow} 0.29) displayed poor adsorption to the PDMS sponge. Caffeine, lincomycin, sulfamethoxazole and trimethoprim had negligible removal by the

PDMS sponge after 24 hours (<5% decrease from initial concentration) and clotrimazole had the greatest removal (87% decrease from initial concentration). Table 4.4 shows the relationship between the tested compounds and their respective Log K_{ow} to the PDMS sponge based on the exponential decay model $C_{water(t)} = C_{water(0)}(e^{-kt})$, where a is the $C_{water(t)}$ is the concentration at time t, $C_{water(0)}$ is the initial concentration, k is the change factor (rate of adsorption), and t is time. The required for the PDMS sponge to adsorb half of the initial concentration ($t_{1/2}$) was calculated based on the equation $t_{1/2} = \ln 2/k$ (Villar et al., 2018).

Table 4.4 Log K_{ow} and its related rate of adsorption, R^2, $t_{1/2}$ and % decrease to the PDMS sponge based on the exponential decay model $C_{water(t)} = C_{water(0)}(e^{-kt})$.

Compound	**Log K_{ow}**	**Rate of adsorption (k)**	**R^2**	**$t_{1/2}$ (hours)**	**% decrease**
Caffeine	0.16	8.174E-13	-7.357E-11	8E+11	4
Lincomycin	0.29	6.88E-13	-1.697E-10	1E+12	-1
Sulfamethoxazole	0.48	0.0090	0.1706	77	2
Trimethoprim	0.73	0.0017	0.0427	400	-1
Norcocaine	1.96	0.0226	0.2791	31	31
Carbamazepine	2.25	0.0261	0.7134	27	49
Diltiazem	2.79	0.0238	0.8204	29	36
Atrazine	2.82	0.0174	0.3479	40	46
Diphenhydramine	3.11	0.0257	0.9005	27	44
Fluoxetine	4.65	0.0616	0.8335	11	74
Sertraline	5.29	0.1335	0.5101	5	54
Clotrimazole	6.26	0.1418	0.7613	5	87

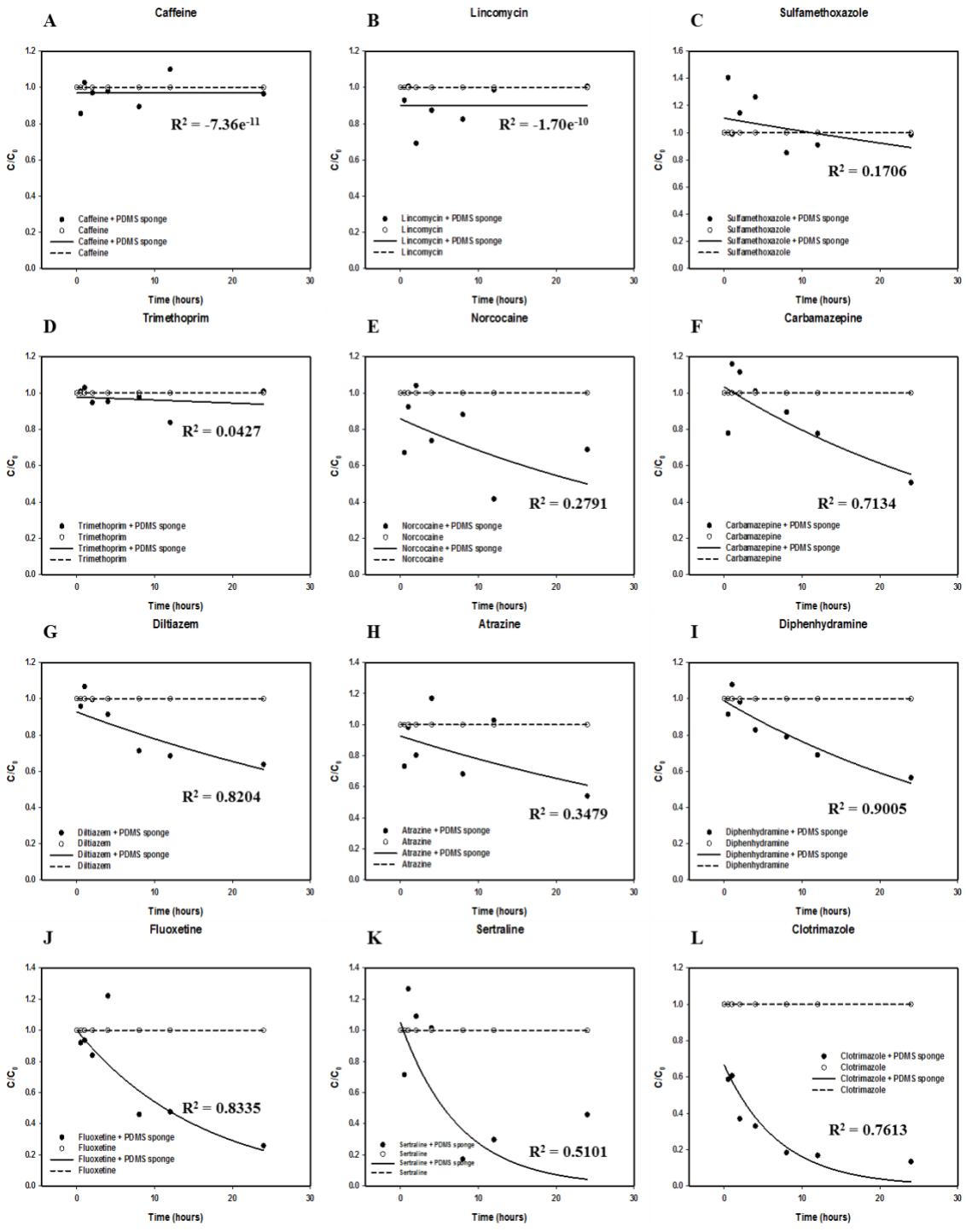

Figure 4.1 Measured peak area in water over time for compounds of varying Log K_{ow}. In order of increasing Log K_{ow} A) caffeine (0.16), B) lincomycin (0.29), C) sulfamethoxazole (0.48), D) trimethoprim (0.73), E) norcocaine (1.96), F) carbamazepine (2.25), G) diltiazem (2.79), H) atrazine (2.82), I) diphenhydramine (3.11), J) fluoxetine (4.65), K) sertraline (5.29), L) clotrimazole (6.26).

Since the adsorption by the PDMS sponge is dependent on the compounds Log K_{ow}, the correlation between them was evaluated and has shown to have a direct relationship and to be linear, with an $R^2 = 0.8252$ (Figure 4.2).

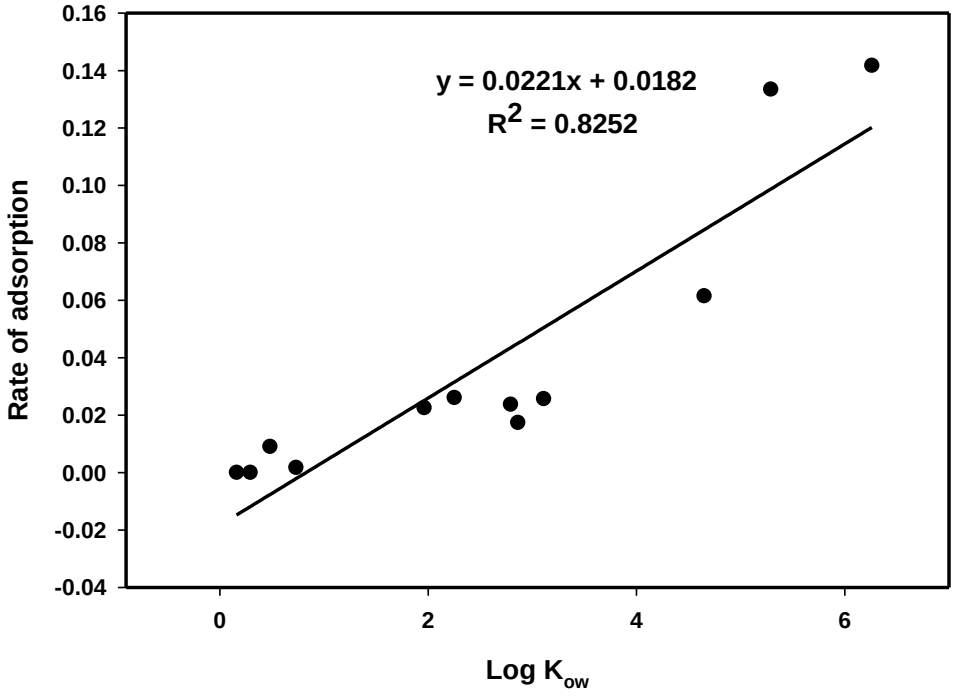

Figure 4.2 Correlation between adsorption rate and Log K_{ow}.

This preference of PDMS towards compounds with higher Log K_{ow} was also observed by (Villar et al., 2018) in which they used PDMS pellets as a passive sampler for dissolved contaminants (organochlorine pesticides and polycyclic aromatic hydrocarbons) in the aquatic environment and by (Nianzhen et al., 2009) where the behavior of 2 compounds with different hydrophobicity with PDMS were studied and it was found that Calcein which is a hydrophilic compound had negligible adsorption to PDMS and 5- (and 6-) carboxytetramethylrhodamine (TMR) which is a more hydrophobic dye adsorbed to

PDMS. In a study by (Bell and Gardinali, 2010) in which they assessed PDMS rods towards the removal of atrazine, one of the compounds tested in this work, and it was found that the PDMS rods removed 30% of atrazine, whereas in this study, the PDMS sponge removed 46%. The increase in performance in the removal of atrazine by the PDMS sponge compared to the PDMS rods can be attributed to the microporous nature of the PDMS sponge composites and increased surface area. This preference and tendency of PDMS to progressively adsorb compounds with increasing Log K_{ow} is due to its hydrophobic and oleophilic properties (Tran et al., 2015). In addition, a molecule's polarity, steric effect, PDMS thickness and surface area, functionality and molecular size of chemical compounds can affect the adsorption process (Theodoridis et al., 2004; Villar et al., 2018; Zeng et al., 2005).

Activated charcoal's adsorptive properties have long been used for the treatment of acute poisoning and for the prevention of absorption of drugs and poisons as the charcoal has a greater affinity to adsorb onto itself (Gude et al., 2010) and have shown to effectively remove a wide range of compounds (Bainbridge et al., 1977; Lin et al., 2019; Malhas et al., 2002; Mor et al., 2017; Neuvonen and Elonen, 1980; Zacaroni et al., 2015). While copper has been shown to have biocidal properties and used for the inactivation of bacteria and virus (Bleichert et al., 2014; Deng et al., 2017). However, an issue with the use of unbound charcoal and copper nanoparticles for remediation is the recovery. Immobilization of these particles onto a substrate such as PDMS can allow the recovery these particles and to be cleaned for reuse. Previous studies have enhanced the function of PDMS through surface modification with graphene (Tran et al., 2015), iron (Bell and Gardinali, 2010), zinc oxide (Michel et al., 2018), carbon nanotubes and titanium dioxide

(Lian et al., 2020). One such study successfully treated wastewater containing rhodamine B dyes using a PDMS microparticles functionalized with carbon nanotubes/titanium dioxide nanocomposites by utilizing the synergistic effect of both sorption and photocatalytic degradation in which the PDMS sorbed the pollutants and the titanium dioxide generated reactive oxygen species for the decomposition of them (Lee et al., 2019; Lian et al., 2020). Another study improved the surface biocompatibility of PDMS for prolonged cell study by coating the PDMS surface with a bio-inspired polydopamine (Chuah et al., 2015). This not only stabilized the adhesion and multipotency of the cell, but also changed the surface wettability. With this in mind, we functionalized the PDMS sponge with activated charcoal to enhance its adsorption capability and with copper to function as a biocide. In this study, it was observed that functionalizing of the PDMS sponge with charcoal improved the adsorption efficiency of the organic compounds for example, 99% of norcocaine and diphenhydramine was removed after 24 hours, an improvement of 68% and 55% respectively, with the PDMS and charcoal sponge requiring only 10 minutes (0.17 hours) to remove half of the initial concentration ($t_{1/2}$) of each compound, compared to the PDMS sponge only which had a removal of 31% and 44% for norcocaine and diphenhydramine, and $t_{1/2}$ of 31 hours and 27 hours respectively (Figure 4.3). Although activated charcoal has the ability to adsorb a wide variety of substances onto its surface, the adsorptive tendencies vary considerably for different organic chemicals and is affected by pH, the properties of both charcoal and organic chemicals, temperature and the presence of competing adsorbing chemicals and/or substances (Le-Minh et al., 2018; Neuvonen, 1982).

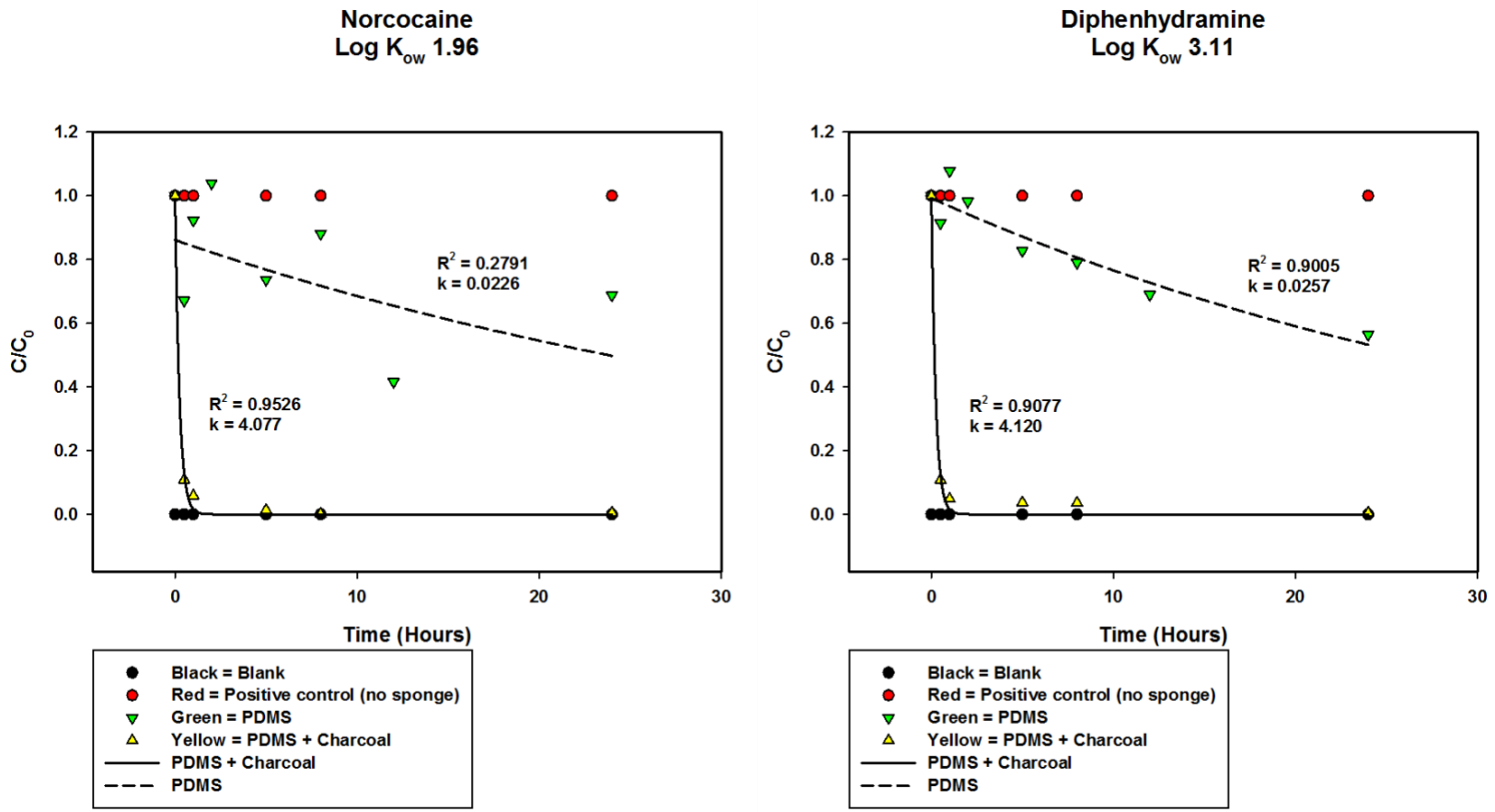

Figure 4.3 Adsorption over time with PDMS sponge functionalized with charcoal in terms of peak area of the studied compounds. A: norcocaine, B: diphenhydramine

Due to the antimicrobial property of metallic copper, bacteria are killed when they come into contact with metallic copper surfaces (contact killing) (Grass et al., 2011). The antibacterial property of metallic copper is as a result of the copper ions being released from the surface which causes oxidative stress and membrane damage (Bleichert et al., 2014; Deng et al., 2017; Grass et al., 2011). Metallic copper has been reported to effectively inactivate both gram negative and gram positive bacteria as well as virus' in a matter of minutes (Bleichert et al., 2014) and the inactivation efficiency is directly related to copper ion concentration (Deng et al., 2017). In addition to functionalizing with charcoal, PDMS sponges were also functionalize with metallic copper to show its versatility and potential towards the inactivation of bacteria. Scanning electron microscope (SEM) images were taken of the copper functionalized sponge and as can be seen in Figure 4.4, the surface of the copper is exposed and accessible to come into contact with *E. coli* if present.

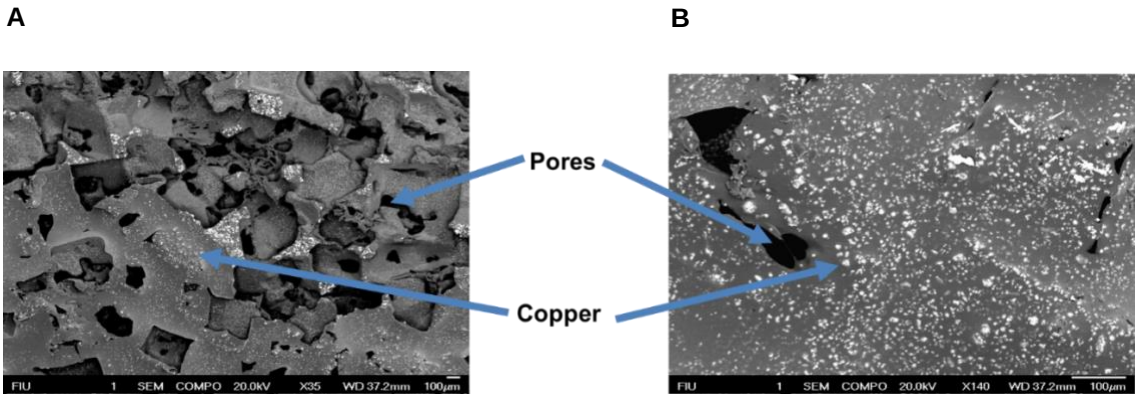

Figure 4.4 Backscatter electron images by field emission SEM of copper functionalized PDMS sponge. A: 35X magnification, B: 140X magnification.

The functionalized copper sponges were applied to environmental samples that were previously known to be contaminated with *E. coli* and it was found that the copper sponge inactivated the amount of *E. coli* by an average of 112 cfu per 100 mL of sample after 4

hours. This was an average of 42% reduction of *E. coli* in the sample with a copper sponge compared to the control sample in which there was no copper sponge as shown in Figure 4.5 and Figure 4.6. Similar inactivation efficiency of *E. coli* was observed by (Deng et al., 2017).

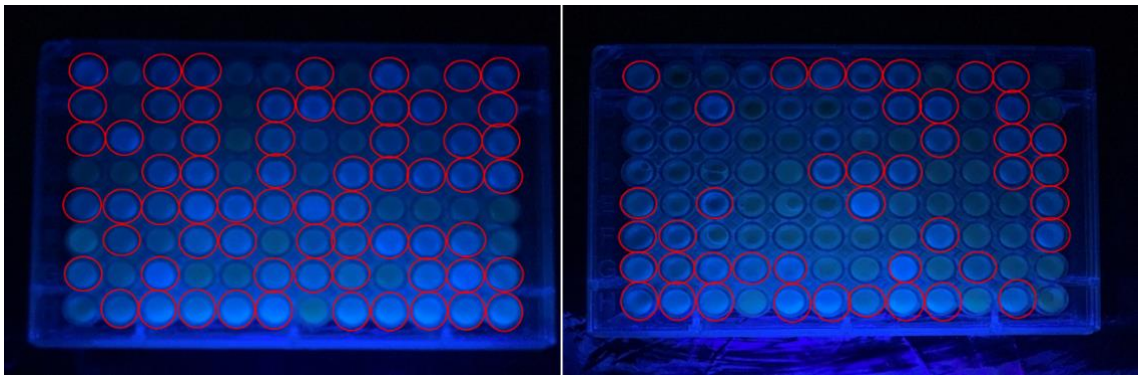

Figure 4.5 E. coli results from Coliplates test of environmental water samples. Left: Control (environmental sample only), Right: environmental sample and copper sponge.

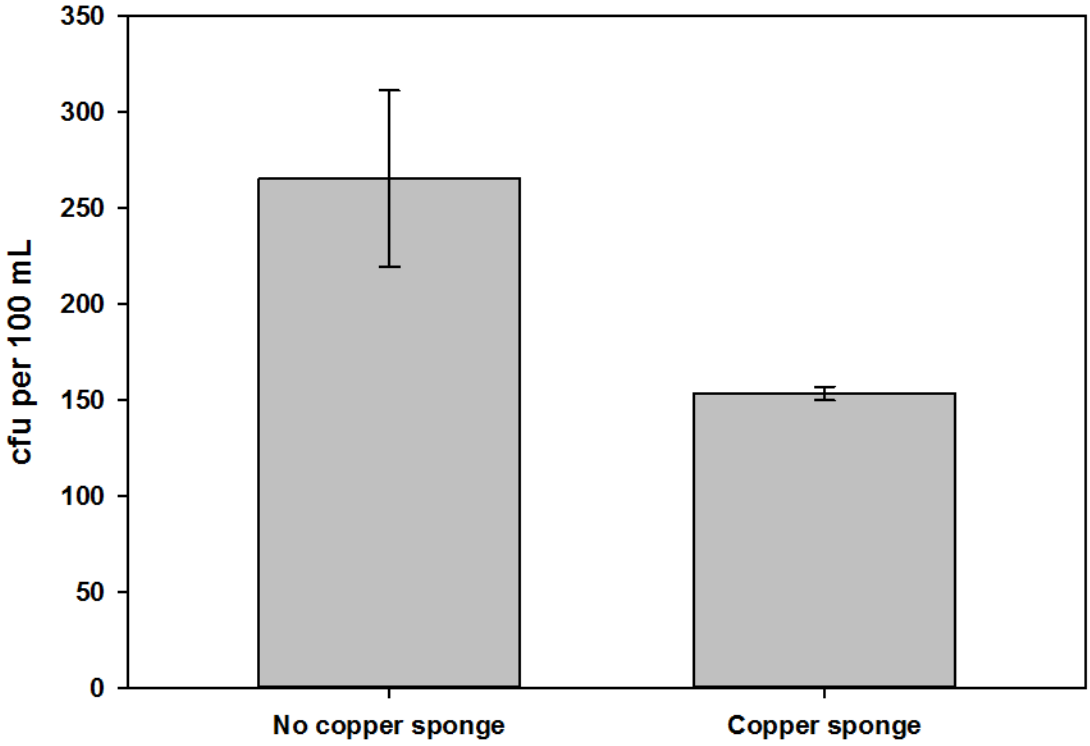

Figure 4.6 The inactivation performance of the copper sponge on *E. coli* in environmental water samples.

4.4 CONCLUSIONS

This study demonstrated the potential of the PDMS sponge as a simple, low cost tool for the removal of organic contaminants in the aquatic environment and its versatility to be modified to not only improve its absorptive capabilities but also function. There is a strong correlation between Log K_{ow} and the adsorption of compounds by the PDMS sponge, with increasing Log K_{ow} having a higher rate of absorption. The correlation between adsorption rate and Log K_{ow} was also evaluated and shown to have a linear relation with a R^2 of 0.8252. The functionalizing of the PDMS sponge with charcoal improves its

ability to remove organic contaminants in the environmental by up to 68% and at a much quicker rate (minutes to remove half the initial concentration). The copper functionalized PDMS sponge reduced the amount of *E. coli* in environmental samples by an average of 42% and can potentially be used for the deactivation of bacteria in the aquatic environment. The work in this study provided the proof of concept in which PDMS sponge composites can be used towards the remediation of urban waters. Future work will involve the scaling up of the developed sponge composites for use in high flow systems as a filter, adsorbent and biocide that can be easily implemented into the real world such as under storm drains or at the end of water outfalls in an urban environment.

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CHAPTER 5 CONCLUSIONS

Objective 1: The benchmarks to assess reproducibility are not well defined for non-target analysis. Parameters to evaluate analytical performance, such as accuracy, precision and selectivity, are well defined for target analysis, but remain elusive for non-target screening analysis. In the first aim of this dissertation, quality control (QC) guidelines were proposed to assure reliable data in non-target screening methodologies using a simple set of standards. Workflow reproducibility was assessed using an in-house QC mixture containing selected compounds with a wide range of polarity that can be detected either by electrospray ionization (ESI) in positive or negative mode. The analysis was performed by online solid phase extraction (SPE) liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Data processing was done by a commercially available software, Compound Discoverer v. 3.0 using an environmental working template, which searched a multitude of databases, including Chemspider, EPA Toxcast, MzCloud among others. We have specifically evaluated method specificity, precision, accuracy and reproducibility in terms of peak area and retention time variability, true positive identification rate, intraday (within days) and interday (consecutive days) variations and the use of QC samples to reduce false positives based on a RT vs Log K_{ow} model. The method showed a satisfactory accuracy with an identification rate of $\geq$ 70% for most of the QC compounds. Precision estimated based on peak area relative standard deviation (RSD) ranged between 30 to 50% for most of the compounds. Data normalization to a single internal standard did not improve peak area variability. Retention time precision showed great repeatability and reproducibility (RSD $\leq$ 5%). In addition, a simple model of RT vs log K_{ow} was designed based on our QC mixtures to efficiently reduced false positives by an average of 49.1% by the elimination of detected features with a Log K_{ow} that has a

retention time that does not fit into this model. In addition, this QC mixture can be used to evaluate instrument and analytical performance before, during and after analytical batch analysis. This QC mixture can be customized as needed and easily implemented into any analytical laboratory.

Objective 2: The development of NTA methods to assess environmental contaminants of emerging concern, which are not commonly monitored for, is of great importance especially when there is no previous knowledge on the identity of the pollution source. In the second aim of this study, we have compared ESI and APCI for the detection and identification of organic contaminants in tap and surface waters from South Florida. In addition, the performance of the developed non-targeted analysis method was evaluated by analyzing 10 complex mixtures as part of the inter-laboratory study ENTACT lead by the U.S. EPA. Different water bodies have shown unique chemical features that can be used as a chemical fingerprint for source tracking and differentiation. APCI has detected at least 3 times as many chemical features as that of ESI in environmental water samples, which corroborates the fact that APCI is more energetic and can ionize certain classes of compounds that are traditionally difficult to ionize in LC-MS, less background and more gas phase reactions. To evaluate the chemical space coverage, kendrick mass defect plots and van Krevelen diagrams of the EPA's DSSTox database were plotted and applied to water samples from South Florida to describe the chemical space covered by ESI and APCI by grouping and presenting unique patterns for compounds belonging to similar classes. Despite the numeric differences, APCI and ESI were found complementary, expanding the NTA chemical space being detected, which would otherwise be underestimated by a single ionization source operated in a single polarity setting.

Objective 3: Lastly, in this dissertation work, we successfully utilize the sorptive properties of PDMS and made it into a sponge which has an increased surface area over a bulk mass of PDMS and applied it towards the removal of organic contaminants as well as functionalized it to improve its adsorption capabilities and biocidal properties. The PDMS sponge was applied towards the removal of a wide range of chemical compounds that cover a wide range of Log K_{ow} (0.16 to 6.26). The PDMS sponge showed greater affinity towards compounds with larger Log K_{ow} and did not adsorb compounds with lower Log K_{ow}, which can be attributed to the hydrophobic and oleophilic nature of the PDMS sponge. This adsorption of the PDMS sponge with Log K_{ow} was found to be linear, with an $R^2 = 0.8252$. The functionalizing of the PDMS sponge with charcoal increased its performance and showed superior adsorption efficiency compared to just plain PDMS in terms of both the amount of organic chemical compounds removed after 24 hours and the time it takes to remove half of the initial concentration. The functionalizing of the PDMS sponge with metallic copper provided antimicrobial properties and reduced the amount of *E. coli* in environmental samples by an average of 42% after 4 hours of contact and can potentially be used for the deactivation of bacteria in the aquatic environment. The work in this study provided the proof of concept in which PDMS sponge composites can be used towards the remediation of urban waters. Future work will involve the scaling up of the developed sponge composites for use in high flow systems as a filter, adsorbent and biocide that can be easily implemented into the real world such as under storm drains or at the end of water outfalls in an urban environment.

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PUBLICATIONS AND PRESENTATIONS

Brian Ng, Natalia Quinete, Stephanie Maldonado, Kathleen Lugo, Julian Purrinos, Henry Briceño, Piero Gardinali. Understanding the occurrence and distribution of emerging pollutants and endocrine disruptors in sensitive South Florida Ecosystems. Sci. Total Environ. 2021, 757, 143720.

Autumn Henderson, Brian Ng, Steven Landeweer, Natalia Quinete, Piero Gardinali. Assessment of sucralose, caffeine and acetaminophen as anthropogenic tracers in aquatic systems across Florida. B. Environ. Contam. Tox. 2020, 105(3) 351-357.

Brian Ng, Natalia Quinete, Piero Gardinali. Assessing accuracy, precision and selectivity using quality controls for non-targeted analysis. Sci. Total Environ. 2020, 713, 136568.

Brian Ng, Natalia Quinete, Piero Gardinali. Comparison of different ionization sources in the detection and identification of organic contaminants in environmental waters by non-targeted analysis. Submitted.

Brian Ng, Natalia Quinete, Piero Gardinali. A simple polydimethylsiloxane (PDMS) sponge for the removal of environmental organic contaminants. In Preparation.

Brian Ng, Natalia Quinete, Stephanie Maldonado, Kathleen Lugo, Julian Purrinos, Henry Briceño, Piero Gardinali. Understanding the occurrence and distribution of emerging pollutants and endocrine disruptors in sensitive coastal South Florida ecosystems. Southeast Florida Coral Reef Initiative (SEFCRI) Technical Advisory Committee Meeting, April 2, 2021. (Invited talk)

Brian Ng, Brunie Gue, Piero Gardinali. Discrimination of soil using complimentary tools: geographical attribution of soils using inductively coupled plasma mass spectrometry and x-ray fluorescence. SETAC North America 41st Annual Meeting, SciCon2, November 15-19, 2020.

Brian Ng, Natalia Quinete, Piero Gardinali. A simple polydimethylsiloxane (PDMS) sponge for the removal of environmental organic contaminants. SETAC North America 40th Annual Meeting, Toronto, Canada, November 5th, 2019.

Brian Ng, Natalia Quinete, Piero Gardinali. Assessing accuracy and precision using quality controls for non-targeted analysis. SETAC North America 40th Annual Meeting, Toronto, Canada, November 5th, 2019.

N. Quinete, K. Lugo, S. Maldonado, B. Ng, H. Briceno, P. Gardinali. Assessment of steroid hormones and pharmaceuticals in South Florida surface waters by liquid chromatography-high resolution mass spectrometry. SETAC North America 40th Annual Meeting, Toronto, Canada, November 4th, 2019.

Brian Ng, Natalia Quinete, Piero Gardinali. Redefining quality controls for non-target analysis of environmental samples. 15th Annual Workshop on LC/MS/MS Applications in Environmental Analysis and Food Safety, Miami, FL, May 29-31, 2019. (Oral Presentation – 2nd place student oral presentation)

Natalia Quinete, Brian Ng, Piero Gardinali. Comparison of different ionization sources in the detection of unknown compounds in environmental samples within the EPA ENTACT project. SETAC North America 39th Annual Meeting, Sacramento, CA, November 6th, 2018. (Oral Presentation)

Brian Ng, Natalia Quinete, Piero Gardinali. Improving non-target analysis by HPLC-ESI/HRMS in the context of the EPA collaborative trial project (ENTACT). SETAC North America 39th Annual Meeting, Sacramento, CA, November 6th, 2018.

Brian Ng, Natalia Quinete, Piero Gardinali. Non-target analysis by high-performance liquid chromatography-electrospray ionization/high resolution mass spectrometry (HPLC-ESI/HRMS) for the detection of "unknowns". EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) Workshop, U.S. Environmental Protection Agency, Durham, NC, August 13-15, 2018.

Natalia Quinete, Brian Ng, Piero Gardinali. Comparison of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in the detection of unknown compounds within the context of the EPA ENTACT project: should ESI be the standard approach in non-targeted analysis? EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) Workshop, U.S. Environmental Protection Agency, Durham, NC, August 13-15, 2018.

Brian Ng, Natalia Quinete, Piero Gardinali. Non-target analysis using online SPE coupled to HPLC-ESI/HRMS as a fingerprinting tool to characterize water sources. SETAC North America 38th Annual Meeting, Minneapolis, MN. November 16th, 2017. (Oral Presentation)