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Development and Validation of Advanced Analytical Methods for Pesticides, Per- and Polyfluoroalkyl Substances, and Opioids in Soil and Drinking Water

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DEVELOPMENT AND VALIDATION OF ADVANCED ANALYTICAL METHODS FOR PESTICIDES, PER- AND POLYFLUOROALKYL SUBSTANCES, AND OPIOIDS IN SOIL AND DRINKING WATER

BY

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DISSERTATION ACCEPTANCE PAGE

Christopher Skaggs

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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This dissertation is dedicated to my loving wife, Lisa, and our two amazing children, Juliette and Soren. I am forever grateful to the three of you and the sacrifices you had to make during my pursuit of this degree. I'm sorry for all the nights and weekends I missed, but know, this it is not my achievement, but **ours**. If any solace can be gleaned from my absenteeism, know that nothing is impossible. I ask that you never underestimate how strong you are, never give up, and never stop believing in yourselves—I know I never will.

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ABBREVIATIONS

GC-MS: Gas chromatography-mass spectrometry

HPLC-MS/MS: High performance liquid chromatography-tandem mass spectrometry

ICECLES: Ice concentration linked with extractive stirrer

LLE: Liquid-liquid extraction

LOD: Limit of detection

LOQ: Limit of quantification

MRM: Multiple reaction monitoring

N-EtFOSAA : N-ethyl perfluorooctane sulfonamideo acetic acid

N-MeFOSAA: N-methyl perfluorooctane sulfonamideo acetic acid

PFBS: perfluorobutanesulfonic acid

PFDA: perfluorodecanoic acid

PFDoA: perfluorododecanoic acid

PFHpA: perfluoroheptanoic acid

PFHxS: perfluorohexanesulfonic acid

PFHxA: perfluorohexanoic acid

PFNA: perflourononanoic acid

PFOS: perfluorooctanesulfonic acid

PFOA: perfluorooctanoic acid

PFTeDA: perfluorotetradecanoic acid

PFTrDA: perfluorotridecanoic acid

PFUdA: perfluoroundecanoic acid

PDMS: Polydimethylsiloxane

SBSE: Stir bar sorptive extraction

SPE: Solid phase extraction

SPME: Solid-phase microextraction

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ABSTRACT

DEVELOPMENT AND VALIDATION OF ADVANCED ANALYTICAL METHODS FOR PESTICIDES, PER- AND POLYFLUOROALKYL SUBSTANCES, AND OPIOIDS IN SOIL AND DRINKING WATER

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Environmental pollution from contaminants is a serious concern in a world where more and more pesticides and pharmaceuticals are being used, sometimes improperly and in excess. Since pesticides are used on practically every crop on Earth, it's no surprise that these compounds are detected in soil, water, and processed agricultural commodities meant for human consumption. Additionally, because of the ubiquity of pharmaceuticals, specifically opioids, and their inherent addictive properties, these compounds are being over-consumed. Because current waste-water treatments can be insufficient to remove them from water sources, this consumption has led to the detection of these compounds in drinking water. Other man-made compounds, including per- and polyfluoroalkyl substances (PFAS), have also been detected in drinking water. There has been evidence of these compounds causing various health issues, including cancer. The ability to detect pesticides, opioids, and PFAS at ultratrace concentrations is vital; therefore, Ice Concentration Linked with Extractive Stirrer (ICECLES) and direct injection, in conjunction with high performance liquid chromatography—tandem quadrupole mass spectrometry (HPLC-MS/MS), were used for their detection at ultratrace levels.

Due to the inherent danger of water and soil contamination with pesticides, PFAS, and opioids, their detection at ultratrace concentrations is vital, as their chronic effect on

human health, even at low concentrations, is relatively unknown. Therefore, several analytical methods were developed, validated, and applied to the analysis of field samples to detect these contaminants in soil and water from across the U.S. For soil, a method was developed for the analysis of atrazine in soil that generated a Limit of Detection (LOD) and Limit of Quantification (LOQ) of 5 and 10 ng/kg, respectively. This method required minimal extraction and generated an LOQ 100x lower than the next most-sensitive method. This soil method was able to detect atrazine in various U.S. soils. For drinking water analysis, a method to detect PFAS was developed. This method required only 50 µL of organic solvent (methanol) for each sample, no SPE cleanup, no filtration and/or evaporation and reconstitution, while generating ultratrace LODs and LOQs for the 14 PFAS tested. This method was then applied to various U.S. drinking water samples and detected values as high as 213 ng/L. The developed opioid method generated LODs and LOQs for all three compounds ranging from 0.15 to 1.5 and 0.5 to 5.0 pg/mL, respectively. After validation, the method was applied to the analysis of various U.S. drinking water samples and detected hydrocodone and codeine with a prevalence of 79%. In addition, there is no previous study reporting opioid concentrations in U.S. drinking water sources, and so this work provides a simple, direct injection analysis for opioids in drinking water not previously reported. Lastly, using atrazine as a probe molecule, an extensive evaluation of ICECLES compared to other techniques, liquid-liquid extraction (LLE), solid phase extraction (SPE), stir bar sorptive extraction (SBSE), and solid phase micro-extraction (SPME), with analysis via HPLC-MS/MS was performed. ICECLES, SBSE, and SPME extractions of five compounds (atrazine, furfural, 2-methylpyrazine, 1-pentanol, and indole) from water were also compared.

Chapter 1. Introduction

1.1 Overall Significance

With the advent of highly selective analysis techniques (e.g., liquid chromatography-tandem mass spectrometry), and lower limits of detection requirements, extraction efficiency is arguably the most important property of modern sample preparation techniques. The determination of contaminants (e.g., pesticides, opioids, or other unwanted analytes) in drinking water, soil, and food is of great importance. The analysis of contaminants at relevant concentrations typically requires highly selective instrumentation, such as GC-MS or HPLC-MS/MS. Even with the use of highly selective analysis techniques, most analyses requiring detection of trace concentrations also necessitate advanced sample preparation techniques to eliminate interferences and to preconcentrate analytes prior to analysis. The most common sample preparation techniques used in modern analytical chemistry include SPE, LLE, SPME and SBSE.[1] While these techniques have proven to be excellent at extracting, purifying, and pre-concentrating many analytes, some analytes, such as pesticides and pharmaceuticals have still proven to be difficult to analyze at ultratrace concentrations.

First demonstrated by Maslamani et al., [2] ICECLES combines the advantages of SBSE and freeze concentration (FC) to create a simple sample preparation technique that is automated, requires no organic solvent if using thermal desorption and only a small amount of organic solvent if back-extracting (e.g., 500 µL or less), separates analytes from matrix interferents, and preconcentrates analytes for analysis. ICECLES provides a simple and automated extraction technique alternative that minimizes matrix interference and preconcentrates analytes. ICECLES has been used to prepare the complex matrix of

green tea for food/flavor analysis, where ICECLES was used to extract over 300 compounds from green tea, 54 more than found in a direct comparison with SBSE study.[3] ICECLES has also been used to successfully detect nitrosodipropylamine, a carcinogen, in drinking water at ultratrace concentrations.[4]

The ICECLES apparatus, including the arrangement of the double-walled glass beaker, is shown in Figure 1.1. The sample solution is progressively frozen from the bottom of the vial to the top via coolant flow (ca. -5 °C) through the double-walled beaker. Samples are initially stirred at a relatively high rate (1200 rpm), and eventually slowed down to approximately 300 rpm once the height of the solution (i.e., the distance between the top of the ice and the top of the solution) decreases to approximately 1 cm to prevent splashing of the concentrated sample solution. The progressive concentration ability of ICECLES has been shown and demonstrates the importance of fully freezing the aqueous sample to ensure maximum analyte migration into the stir bar. The extracting power of ICECLES increases as the liquid sample freezes. Following ICECLES, stir bars are analyzed directly via TD-GC-MS or placed into a clean vial and back-extracted for analysis via HPLC-MS/MS.

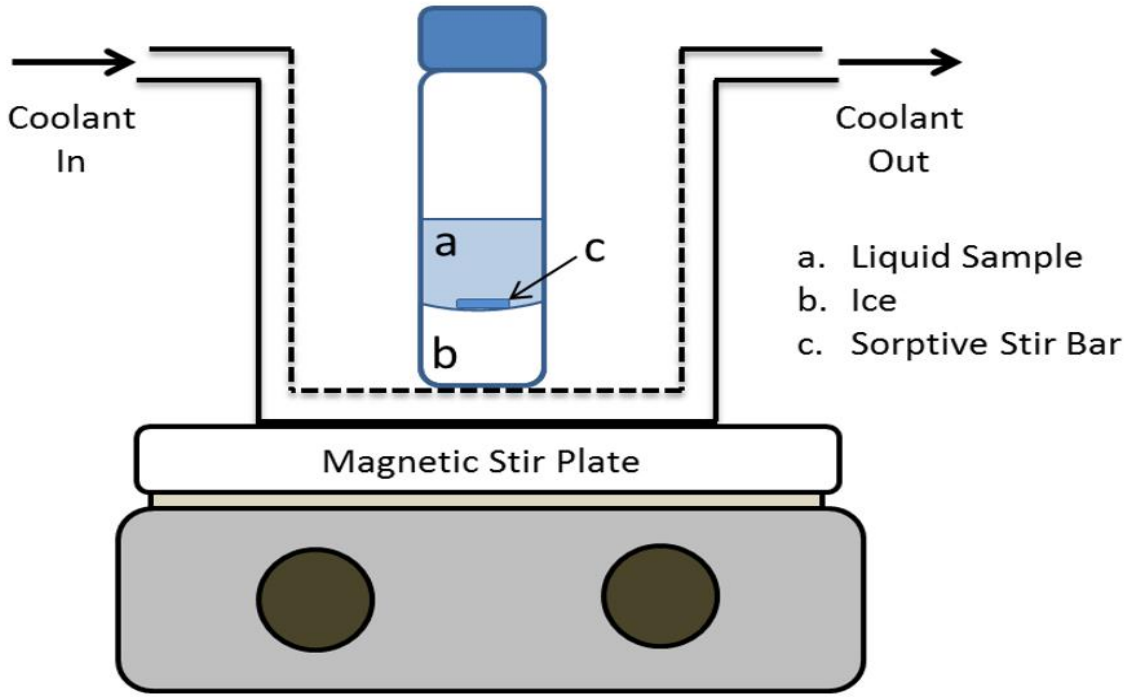


Figure 1.1 Diagram of the ICECLES apparatus displaying the flow of coolant through a double-glass beaker, which cools and slowly freezes an aqueous sample from the bottom to the top of the vial while the stir bar spins.

1.2 Objectives

This work consists of four main objectives: (1) develop an ICECLES technique to extract atrazine from soil (Chapter 2), (2) compare common extractive sample preparation techniques to ICECLES (Chapter 3), (3) use ICECLES to extract PFAS from drinking water (Chapter 4) and (4) extract opioids from drinking water (Chapter 5). The first objective consists of a method that was developed using ICECLES to extract atrazine from soil. The second objective demonstrates the robustness of ICECLES compared to other analytical techniques, compares various other sample preparation techniques (i.e., SBSE, SPME, LLE, and SPE). The extraction efficiency of all of these techniques were compared using dozens of compounds, including pesticides and aromas/flavors from pure and drinking water to demonstrate the ability of ICECLES to provide an alternative to legacy extraction techniques, especially for more polar compounds. For the third objective, we used ICECLES to extract 14 PFAS from drinking water with only 10 mL of sample and was able to minimize the amount of organic solvent required for each sample versus current methods (e.g., SPE-LC-MS/MS) by up to 1,200x. For the fourth objective, direct injection analysis was utilized to analyze opioids from drinking water. Due to a paucity of publications and information in the literature, this method was able to demonstrate for the first time, the detection of hydrocodone and codeine in U.S. water sources.

1.3 Environmental Contamination

Environmental contamination and pollution are unfortunately not new phenomena, but something mankind has exacerbated with the development of pesticides, pharmaceuticals, and fluorinated compounds (PFAS). These three particular classes of compounds are some of the most prevalent environmental contamination because of their ubiquity and overuse. Though these compounds are very useful (e.g., pesticides allow for greater yields of crops, pharmaceuticals ease pain and treat disease, and PFAS have improved our quality of life for everything from cooking to cell phones), compound properties, excess consumption, and improper disposal has contaminated our environment.

1.3.1 Water and Soil Contamination

Two of the most serious environmental concerns are those of water and soil contamination. Water, of course, is required for all life, and essentially so is soil, as all crops require it for growth. When it comes to water contamination, we must also consider the ecosystems that can be negatively affected by these compounds (e.g., pesticide poisoning of fish and other aquatic life after heavy rains wash-off excess pesticides from fields). For instance, atrazine is the most detected pesticides found in contaminated drinking water[5] and with a half-life of up to 261 days,[6] it stands to reason that it can affect our water and soil long after it served its initial purpose.

1.3.2 Health Risks

The idea of water and soil contamination should be taken seriously, because as these two matrices become compromised, our health can also be subjected to unknown deleterious effects. For instance, a study[7] from the U.S. Department of Health and Human

Services (HHS) showed how PFAS led to several health issues, including high cholesterol, tumor development, and cancer. Although PFAS are found in many products (cookware, furniture, firefighting foam, electronic devices), these items are not consumed. However, when compounds such as PFAS find their way into our water sources, because of their extremely high bio-persistence, they can then become much more of a threat to our health.

1.3.3 Classification of Pesticides

Since 1975, the World Health Organization (WHO) has been classifying pesticides for the easy determination of their inherent hazards. The WHO has classified pesticides under five categories/classes: Class Ia, Extremely Hazardous (Category 1), Class Ib, Highly Hazardous (Category 2), Class II, Moderately Hazardous (Category 3), Class III, Slightly Hazardous (Category 4), and "Active ingredients unlikely to present acute hazard in normal use (Category 5)."[8] This classification scheme is based on the LD_{50} in rats for each pesticide. Per the WHO's classification scheme[8], these values range from < 5 (Category 1) to 2000-5000 mg/kg bw (Category 5). Additionally, the WHO classifies pesticides via an "9-column" system. This consists of organizing pesticides by their common name, CAS Registry number, UN number[9] (e.g., refers to the UN Recommendations on the transport of dangerous goods), chemical type, physical state, primary use, GHS[10] ("*The Globally Harmonized System of Classification and Labelling of Chemicals*"), the LD_{50}, and remarks.

Depending on who you ask and the source of information, pesticides can be classified into several different groups, all dependent on their target. For instance, the most widely used classifications are fungicides, herbicides, and insecticides, which target

fungi, weeds, and insects, respectively. These three classes constitute the majority of pesticide use; however, there are still very specific classes of pesticides that do not fall into these three categories, for instance, avicides (pesticides that target birds) and rodenticides (pesticides that target rodents).

1.3.4 History and Use of Pesticides

It is believed that humans have been using agricultural practices for approximately 10,000 years[11], beginning in Mesopotamia (present day Jordan, Syria, and Turkey). Similar to farmers today, farmers of the past would have taken steps to help prevent crop loss, and it's believed that the first documented use of pesticides (specifically sulfur compounds) was approximately 4500 years ago.[12, 13]

The 20th century brought on the "golden age" of pesticides; specifically, the synthesis of hundreds of pesticides, ranging from DDT in the 1930s to the newest class of insecticides, neonicotinoids, in the 1990s. Not only have pesticides been developed for agricultural purposes, but for residential needs, such as gardening and pest control (i.e., mosquito spray and/or mouse traps). The EPA estimates that approximately 5.6 billion pounds are used yearly around the world.[14] In the U.S., the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) requires that the EPA ensure that when a pesticide is used according to its label, it won't cause harm to the environment or health, with reasonable certainty.[15]

Pesticides are an extremely valuable tool to protect crops against pests, weeds, and disease. When being applied to crops, pesticides inevitably find their way into the topsoil and can dissipate to lower horizons, where they can accumulate over time. The ultratrace determination of pesticides in soil is vital to understanding the soil's health and

to determining if these residues are causing adverse effects. Soil is the common denominator for all crop growth; therefore, the ultratrace analysis of pesticides are becoming increasingly more needed as regulatory agencies are demanding lower detection limits in water, soil, and crops.[16] Because of their consistent use in modern agriculture, pesticides are also found in surrounding waterways, due to drift, improper spraying, or run-off because of heavy rain events.

1.3.5 Atrazine

Atrazine is one of the most widely used herbicides in the world. Its applicability is wide and can be used on a plethora of crops, but is primarily used on corn, sorghum, and sugarcane. On average, 72 million pounds of atrazine are applied in the U.S. annually,[17] with over 100 pesticide products containing atrazine. It is also applied, to a lesser extent, to residential lawns and golf courses. With such a long half-life as mentioned above, its biopersistence has not gone unnoticed. Specifically, in 2004, the European Union banned atrazine[18] when they found groundwater levels that exceeded regulatory limits and the manufacturer of atrazine, Syngenta, could not demonstrate a means of prevention or that the levels detected were safe. Atrazine moves quickly through plants after being absorbed in the roots. It accumulates in the new leaves of the plant and acts as a photosynthesis inhibitor, ultimately leading to the death of the plant.[19]

1.3.5.1 Structure and Properties

Atrazine is of the triazine herbicide class. Triazines are defined by their nitrogen-containing heterocycles. See Figure 1.2 for atrazine's structure. Atrazine's chemical formula is $C_8H_{14}ClN_5$ with a log K_{ow} of 2.6. Atrazine is practically insoluble in water

from its neat/solid form; however, once in solution in organic solvent (acetonitrile or methanol), atrazine has no issue staying in aqueous solutions.

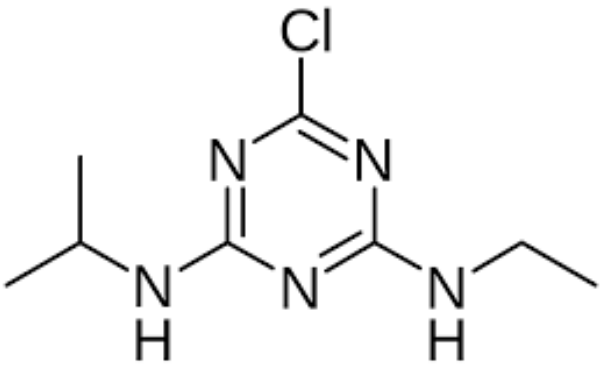


Figure 1.2 Chemical structure of atrazine.

1.3.6 Classification of PFAS

PFAS are man-made chemicals that have been in use in the United States since the 1940s. They are found in food containers, household products (Teflon, polishes, paints, cleaning products, etc..), drinking water (tap, bottled, surface, well), soil, food packaging and living organisms. The EPA's official PFAS method (Method 537.1) lists the following as the most common and important PFAS for which to analyze: N-ethyl perfluorooctane sulfonamideo acetic acid (N-EtFOSAA), N-methyl perfluorooctane sulfonamideo acetic acid (N-MeFOSAA), perfluorobutanesulfonic acid (PFBS), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonic acid (PFHxS), perfluorohexanoic acid (PFHxA), perflourononanoic acid (PFNA), perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorotetradecanoic acid (PFTeDA), perfluorotridecanoic acid (PFTrDA), and perfluoroundecanoic acid (PFUdA).

1.3.7 History and Use of PFAS

Since their creation in the 1940s, PFAS have been utilized in various categories: fluoropolymers, fluororepellants, and fluorosurfactants.[20] Additionally, PFAS are found in medical devices, electronics (cell phones), non-stick cookware, carpet, Class B firefighting foam, and furniture upholstery. Since the early 2000s, the EPA, as well as several industry partners who produce PFAS, agreed that the discontinuation of certain PFAS was necessary. These "long-chained" PFAS were discontinued and replaced with "short-chained" versions, in an effort to generate similar properties, without such lengthy bio-persistence. This is of great concern, as the general population can be exposed to these compounds through indoor environments, food, and water.[21-30]

1.3.8 PFAS

Because PFAS are used in so many applications, their prevalence cannot be overstated. Additionally, because of their ability to biopersist and bioaccumulate, they have drawn the attention of several regulators and concerned groups. Specifically, the EPA developed a lifetime health advisory level for two of the more prevalent PFAS, PFOA and PFOS, at 70 ng/L in drinking water.[31]

1.3.8.1 Structures and Properties

All PFAS have a similar backbone structure (Figure 1.3), characterized by a chain of carbon atoms bonded to fluorine atoms. Other than the difference in carbon atoms, some PFAS also contain functional groups at the end of the chain, such as a sulfonic acid in the case of PFOS. In PFAS, all carbons are attached to fluorine atoms, except for the last one, which is attached to a functional group. Specifically, perfluorinated substances

have all hydrogens on the carbons replaced by fluorines, while polyfluorinated substances have some, but not all, hydrogens on the carbons replaced by fluorines.

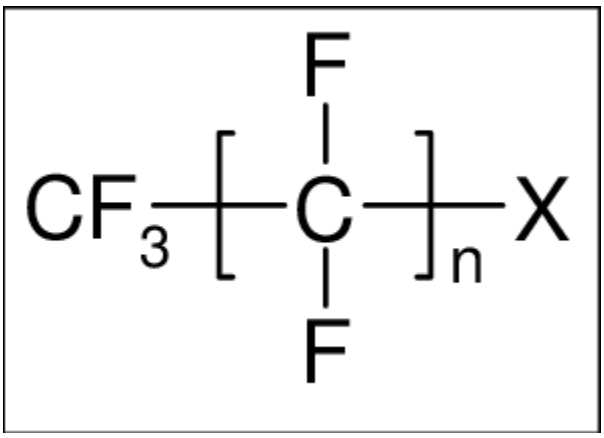


Figure 1.3 Primary backbone structure of PFAS.

Regarding polarity, PFAS vary widely, with the most polar in this work being PFBS (log K_{ow} = 1.8), and the most non-polar being PFDoA (log K_{ow} = 6.9). This trend in polarity follows the number of carbon atoms present in each compound (i.e., PFBS has 4 carbons, while PFDoA contains 12 carbons). Table 1.1 displays the log K_{ow}s and chemical formulas for the PFAS tested in this work.

Table 1.1 Comparison of log K_{ow}s and chemical formulas for PFAS.

Acronym	Log K_{ow}	Formula
PFBS	1.8	$C_4HF_9O_3S$
PFHxS	3.2	$C_6HF_{13}O_3S$
PFHxA	3.5	$C_6HF_{11}O_2$
PFHpA	4.2	$C_7HF_{13}O_2$
PFOS	4.5	$C_8HF_{17}O_3S$
PFOA	4.8	$C_8HF_{15}O_2$
NMeFOSAA	4.9	$C_{11}H_6F_{17}NO_4S$
NEtFOSAA	5.0	$C_{10}H_6F_{17}NO_2S$
PFDA	5.5	$C_{10}HF_{19}O_2$
PFNA	5.5	$C_9HF_{17}O_2$
PFTrDA	6.1	$C_{13}HF_{25}O_2$
PFTA	6.6	$C_{14}HF_{27}O_2$
PFUdA	6.8	$C_{11}HF_{21}O_2$
PFDoA	6.9	$C_{12}HF_{23}O_2$

1.3.9 Classification of Opioids

Opioids are a class of analgesic drugs that react with particular receptors in the body. Three receptors have been shown to have the most interaction with opioids: delta opioid receptor (DOP), kappa opioid receptor (KOP), and mu opioid receptor (MOP).[32] Opioids are classified by what effect they have on receptors; specifically, if they act as agonists, partial agonists, or antagonists. Agonists will produce a maximum desired effect, partial agonists a partial effect, and antagonists bind to receptors, but generate no functional response.[32]

Opioids are also classified by their occurrence, be it natural or synthetic. Compounds such as morphine and codeine occur naturally and can be purified from plants, specifically opium poppies; whereas, compounds such as hydrocodone, fentanyl, and oxycodone are synthetic. Opioids are defined as Schedule II controlled substances per the Controlled Substances Act (CSA).

1.3.10 History and Use of Opioids

Opioids have been used by mankind for thousands of years for medicinal, religious and recreational activities. The earliest mention of using opium as a medicine comes from Homer's Odyssey, whereas priests and shamans have eaten plants to induce trances for millennia.[33]

Modern opioid use arguably began in 1805, when Austrian pharmacist Friedrich Serturner successfully isolated and extracted morphine crystals from the tarry poppy seed juice.[34] After publication in 1805, Serturner performed several experiments on himself and volunteers to indeed demonstrate the pain relieving effects of morphine. He suggested that 15 mg of morphine was the optimal dose and called it "Morphium" after the Greek god of sleep.[34] It would take nearly 150 years (1952) until morphine was synthesized in a laboratory by Marshall Gates.[34]

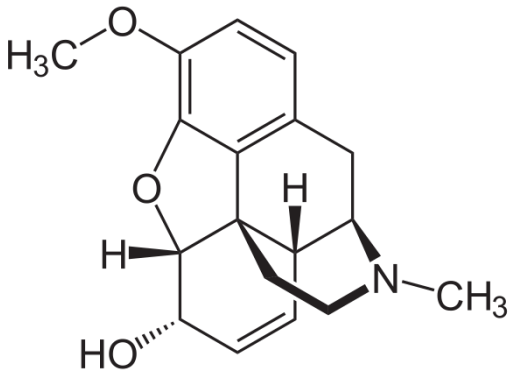
After Serturner's revolutionary discovery, the branch of pharmacology accelerated through the 19th and 20th centuries to present day. In 1832, French chemist Pierre Robiquet was the first to isolate codeine from opium poppies. Codeine is now the most widely used opioid in the world,[35] with approximately 360,000 kg produced every year. After being patented in 1923, hydrocodone has also become a widely used opioid, especially in the U.S., and similar to codeine, is also made from the opium poppy after it has been converted to codeine.[36] First made by Paul Jannsen in 1960, and approved by the U.S. FDA in 1968, fentanyl is a synthetic opioid that has gained unfortunate popularity recently—specifically, because of its highly addictive nature and for being the cause of over 20,000 overdose deaths in the US in 2016.[37, 38]

1.3.11 Opioids

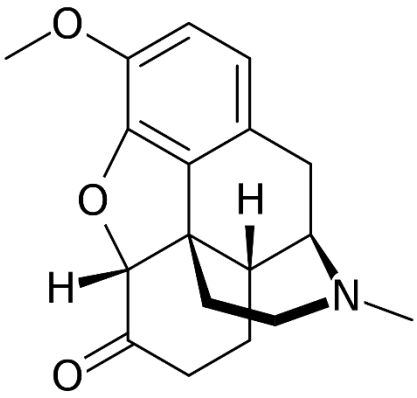
The three opioids studied in this work, fentanyl, hydrocodone, and codeine are all used for pain relief and management. Opioids excel at pain relief because of their ability to act upon and suppress pain receptors in the body. Unfortunately, due to their proficiency at relieving pain, they are also candidates of misuse and abuse, which can be fatal. Fentanyl, for instance, has a lethal dose of only 2 mg in humans—that's 90x more potent than morphine.[37]

1.3.11.1 Structures and Properties

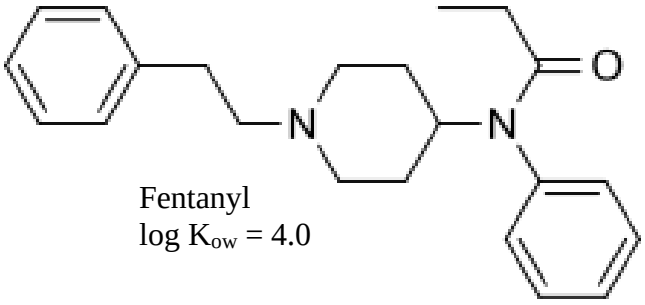
Figure 1.4 shows the chemical structures of fentanyl, hydrocodone, and codeine, as well as their log K_{ow}s. Because of their increased polarity, both hydrocodone and codeine are more soluble in water compared to fentanyl.



Codeine
log K_{ow} = 1.1



Hydrocodone
log K_{ow} = 2.1



Fentanyl
log K_{ow} = 4.0

Figure 1.4 Chemical structures for codeine, hydrocodone, and fentanyl.

1.4 Analysis of Contaminated Soil and Water

Because of the contamination of our soil and water sources, analysis of these contaminants, such as pesticides, PFAS, and opioids, is very important. Detecting these contaminants in both our soil and water is vital to protecting human health. Water is especially important, simply because of the large amounts of it that we consume. Even at low concentrations, chronic exposure over a lifetime could be detrimental. In order to analyze for these contaminants, highly sensitive instrumentation and extraction techniques are needed.

1.4.1 Liquid Chromatography—Tandem Mass Spectrometry (LC-MS/MS)

Liquid chromatography paired with tandem mass spectrometry offers a highly selective and sensitive method for detecting compounds in water and soil at ultratrace concentrations. Liquid chromatography uses pumps and mobile phases consisting of chemical gradients (i.e. aqueous and organic solvents) to separate compounds of interest from interferents on a solid stationary phase (e.g., LC column). Retention and eventual elution of analytes from the LC column are based on their polarity and affinity to the stationary phase. For instance, in reversed phase liquid chromatography, the stationary phase is more non-polar (e.g. C18), and the polarity of the mobile phases usually start with high aqueous concentrations and ramp to higher organic solvent concentrations while simultaneously decreasing aqueous concentration. This gradient allows for the separation of analytes based on their polarity by removing polar analytes from the stationary phase quickly, allowing the more non-polar species to retain on the stationary phase for a time, before ultimately eluting the non-polar species. Once eluted, the sample is transported to a mass spectrometer for detection. In this work, a tandem mass

spectrometer (MS/MS) was used to generated even greater selectivity and sensitivity. The MS/MS contains three major compartments post-source: two mass filters (Q1 and Q3) and a collision cell (Q2) that causes fragmentations as ions collide with gas. Once it reaches the mass spectrometer's source, the liquid sample is desolvated with heat and high voltage through successive Coulombic explosions (i.e., concentrating the analyte through the removal of liquid), until it's nebulized into an aerosol and pulled into the mass spectrometry via vacuum. The mass spectrometer sorts ions based on their masses or mass-to-charge ratio (*m/z*). Once the sample vapor is introduced into the MS, it is ionized, and the decomposed ions are analyzed to produce mass spectra.

1.4.2 Thermal Desorption-Gas Chromatography-Mass Spectrometry (TD-GC-MS)

Thermal Desorption (TD) is a sample preparation method prior to analysis via GC-MS. The TD unit uses heat to desorb analytes from liquids, solids, and stir bars, and the resulting gas is then transported down the GC column via a carrier gas such as helium, nitrogen, or hydrogen. Analytes are then separated on the GC column based on temperature, stationary phase, polarity, and flow rate. Following separation, the analytes are introduced through an interface into the mass spectrometer. At this point, the same principles explained in 1.4.1 are applied; however, for GC-MS, there is no Q3. Instead masses are only filtered in Q1. For GC-MS, there are two common ionization techniques: electron impact (EI) and chemical ionization (CI). EI is considered a "hard" ionization, because it delivers higher energy and causes a large amount of fragmentation, whereas CI delivers less energy and causes less fragmentation.

1.4.3 Sample Preparation Techniques for Ultratrace Analysis

Ultratrace analysis of water and soil typically require some type of sample preparation and/or extraction to help isolate the analytes of interest from interferents. Some methods utilize partitioning into liquids based on polarity, while others use solids such as cartridges and stir bars to desorb analytes from matrices.

1.4.3.1 Liquid-Liquid Extraction (LLE)

LLE is considered a legacy technique as it has been used by chemists for a very long time. LLE works on the theory of partitioning; specifically, two immiscible liquids are combined in a vessel and the analyte is partitioned between the solvents based on the analyte's polarity and intermolecular interactions with the solvent. LLE is simple and easily learned; however, it requires large volumes of organic solvents, specialized glassware (e.g., separatory funnels), lengthy extraction times, and is very labor intensive. LLE is also less suitable for most polar analytes, as these analytes will typically remain in the aqueous layer.

1.4.3.2 Solid-Phase Extraction (SPE)

SPE is a sample preparation technique that exploits the same chemical properties that LLE does; however, instead of partitioning analytes between two liquids, analytes from a liquid are partitioned onto a solid sorbent material. SPE typically utilizes four steps: (1) conditioning the cartridge/sorbent with solvent (methanol and water are the most common), (2) loading the sample onto the sorbent, (3) washing the sorbent material with a solvent that elutes the analytes that are not desired and discarding this eluant, (4) eluting the analytes of interest with a solvent they are soluble in and collecting this eluant for further analysis. Additionally, after elution, most methods require a solvent exchange

(i.e., evaporation of solvent and reconstitution with a more instrument-amenable solvent). SPE has the advantage of multiple sorbent choices, allowing tunable selectivity. This could also be considered a disadvantage for some applications since there is no "one-sorbent retains all" for SPE. Other disadvantages include costly single-use SPE cartridges and elution solvents that may be hazardous. As mentioned previously, SPE requires a sorbent cartridge for each sample analyzed. These cartridges are costly and non-reusable, currently averaging $3 each and are selective for compound properties (e.g. hydrophobicity). Typically, SPE is not automated, making it labor intensive, requiring constant monitoring with multiple steps (conditioning, loading, washing and eluting) and significantly more solvent use than ICECLES. This produces more waste with associated disposal costs. As with LLE, for moderately polar compounds, SPE typically requires very large sample volumes (e.g. up to 1000 mL).

1.4.3.3 Solid Phase Microextraction (SPME)

First demonstrated by Eisert and Pawliszyn,[39] SPME, like SPE, requires a sorbent that extracts compounds from liquid samples (i.e., direct immersion) or headspace. The sorbent is coated on a fiber within a syringe. SPME is a very simple, efficient, and solventless sample preparation method that relies on the partitioning of compounds from the sample to the fiber coating of the syringe. A disadvantage of SPME, however, is the idea of "displacement", where higher molecular weight compounds displace lower molecular weight compounds because of competition for active sites on the fiber coating. Additionally, according to Ouyang,[40] SPME is a non-exhaustive technique where only a fraction of the target analyte is extracted from the sample. As stated above, SPME is also a solventless sample preparation method that partitions/adsorbs compounds from a

sample to a sorbent coating on a fiber. It is an automated technique, as the fiber is directly immersed into the sample or suspended in the headspace and removed after a specified amount of time (i.e. once equilibrium has been reached). However, preparing a batch of samples (i.e. 12 aqueous extracts) for SPME can be cumbersome. Each sample requires its own syringe and PDMS fiber, so analyzing large quantities of samples is relatively expensive. Currently, syringe assemblies cost $450-500 each. SPME also has a relatively low phase ratio (β) compared to sorptive stir bars by a factor of about 100.[41] Therefore, less analyte can be adsorbed into the SPME fiber and it is generally considered non-exhaustive.

1.4.3.4 Stir Bar Sorptive Extraction (SBSE)

SBSE, first introduced by Baltussen, et al,[42] is a solventless sample preparation technique where a sorbent, most commonly polydimethylsiloxane (PDMS), coated on a magnetic stir bar, is placed in a vessel containing a liquid sample and stirred for some time to allow sorption of the analyte on the stir bar coating. After stirring, the stir bar is removed from the samples and either placed into a TDU (Thermal Desorption Unit) for thermal extraction or back-extracted with an amenable solvent, with TDU extraction generally more advantageous, as the entire extract in the stir bar can be analyzed. Despite many advantages, due to the hydrophobicity of PDMS, SBSE is not effective for analytes with relatively low K_{ow} values ($\log K_{ow} < 3$).[43] SBSE is advantageous when analytes have sufficiently high affinities for the stir bar, compared to the solvent. This allows for considerable concentrating of the analyte into the small area of the stir bar. However, a major disadvantage of SBSE (with PDMS-coated stir bars) is that it's typically only

effective for compounds with relatively high log K_{ow} values (log $K_{ow} \geq 3$). Therefore, more polar compounds are typically not effectively adsorbed onto the stir bar via SBSE.

1.4.3.5 Freeze Concentration (FC)

FC is a little-known sample concentration technique that works on the principle of freezing point depression to separate analytes of concern (solute) from matrix interferents (solution) as a solvent is frozen.[44] The aqueous sample is slowly frozen and provides an alternative concentration technique for compounds that are heat-sensitive or volatile and may otherwise be lost. FC forces compounds into the stir bar, whereas with SBSE, only sorption is at work.

1.4.3.6 Ice Concentration Linked with Extractive Stirrer (ICECLES)

First demonstrated by Maslamani,[45] ICECLES combines the advantages of SBSE and FC to create a simple sample preparation technique that is automated, requires negligible amounts of organic solvent (if any) and can separate an analyte from matrix interferents, all while concentrating the analyte of concern for ultratrace detection and comprehensive analysis of mixtures (e.g. food/flavor analysis). This is especially important for compounds which are relatively polar (log $K_{ow} \leq 3.0$).

ICECLES has several advantages, including the ability to concentrate a much wider range of compounds than SBSE, much lower LODs for some compounds versus other common sample preparation techniques and wider applicability of the technique to more volatile and thermally labile analytes. Compared to LLE, ICECLES requires relatively low solvent volumes (i.e., 10 mL of sample) and when back-extracting, ≈50 µL of methanol can be used. Compared to SBSE, ICECLES is very effective at recovering relatively polar compounds (log $K_{ow} \leq 3$). Compared to SPE, ICECLES utilizes re-usable

consumables (stir bars), instead of one-use cartridges. Compared to SPME, the biggest advantage of ICECLES is its high concentration factors.

1.5 Research Goal

The ultratrace analysis of some contaminants in soil and drinking water is both difficult and extremely important for human and environmental health. Since two of our most valuable resources, water and soil, are subjected to constant contamination from pesticides, PFAS, and opioids, this research was performed to develop, validate, and apply analytical extraction techniques to determine contaminants at ultratrace concentrations. In this work, ICECLES and direct injection techniques, utilizing both LC-MS/MS and GC-MS, were evaluated for the analysis of these contaminants from drinking water and soil.

Chapter 2. Development and validation of an HPLC-MS/MS method for atrazine in soil using Ice Concentration Linked with Extractive Stirrer (ICECLES)

2.1 Introduction

Atrazine is a widely-used pesticide with a relatively long half-life in the environment. This leads to persistent soil contamination with the potential of migration to ground and surface waters. Analysis of atrazine in soil is difficult due to the inherent complexity of soil as a sample matrix. Moreover, the moderate hydrophobicity of atrazine makes it difficult to extract into typical sorbent phases during sample preparation. Therefore, a method for the ultratrace determination of atrazine in soil using Ice Concentration Linked with Extractive Stirrer (ICECLES) and high performance liquid chromatography—tandem mass spectrometry (HPLC-MS/MS) was developed to address these issues. For the method, soil samples (10 g) were initially extracted with methanol:water (8:2, v:v), followed by solvent exchange to 100% water. The samples then underwent ICECLES with back-extraction into 100% methanol prior to HPLC-MS/MS analysis. The ICECLES-HPLC/MS/MS method produced a wide linear range of 10 to 1000 ng/kg, featured excellent limits of quantification and detection of 10 and 5 ng/kg, respectively, and good accuracy (100 ± 12%) and precision (≤9.6% relative standard deviation). This method was tested on field soil samples and provided ultratrace detection of atrazine. With this method, previously unachievable low parts per trillion (ppt) detection of atrazine in soil is now possible.

Pesticides are valuable tools for protecting crops against pests, weeds, and diseases. When applied to crops, pesticides commonly infiltrate the topsoil and dissipate

to lower soil horizons, where they can accumulate over time. Therefore, the determination of pesticide residues in soil is vital to ascertaining the soil's health and for understanding if these pesticides are responsible for adverse effects. Because pesticides reside in soil at low concentrations and regulatory agencies are demanding lower detection limits for soil matrices,[16] the ultratrace analysis of pesticides is becoming increasingly important.

Since its creation in 1958 by the Geigy Laboratory, atrazine has become one of the most widely used herbicides globally. It is commonly applied to stop pre- and post-emergence of grassy and broadleaf weeds in such crops as corn, sorghum, and sugarcane. According to the EPA, approximately 72 million pounds of atrazine is applied in the U.S. annually.[17] Due to its popularity, and relatively long half-life, 13 to 261 days[6] depending on the soil and microbial environment, atrazine is consistently found in soil in regions where it is applied. This leads to potential migration of atrazine from soil to ground and surface water, where it can affect human health. In fact, atrazine is the most detected pesticide found in contaminated drinking water.[5]

In order to analyze atrazine from soil at ultratrace levels, extraction is necessary. Legacy methods for extracting atrazine in soil, such as liquid-liquid or Soxhlet[46] extractions, are arduous and typically require large volumes of organic solvent, including halogenated solvents such as dichloromethane, and may also include refluxing and/or hydrolysis. Techniques such as supercritical fluid and accelerated solvent extraction[47, 48] have been presented as alternatives to legacy techniques for soil extraction. While these methods have advantages, they typically require long extraction times, specific equipment and training, and are not amenable to trace analysis. More recently, a group of

advanced extraction techniques have been developed and utilized to extract atrazine from soil. Table 2.5 lists some of these methods for extraction and analysis of atrazine from soil samples with important characteristics of each method reported. Ultrasonic solvent extraction (USE) has been used alone and in combination with solid-phase microextraction (SPME)[49] to extract atrazine from soil. USE-SPME with gas chromatography-electron capture detection (GC-ECD) generated a poor LLOQ (100 µg/kg), while USE alone produced a better LLOQ of 5.0 µg/kg, but poor accuracy.[50] While the LLOQ of the USE method is comparable to the more sensitive techniques in Table 2.5, it cannot be considered ultratrace (i.e., ng/kg LLOQs). More recent methods have utilized QuEChERS to extract atrazine from soil. QuEChERS is a robust and rugged extraction technique that extracts compounds from matrices with the aid of acetonitrile and subsequent salt dehydration; however, because of the typical dilution of analytes in QuEChERS extractions, LLOQs can be higher than desired. The use of QuEChERS was reported in two different studies for the extraction of atrazine from soil (among other compounds), producing LLOQs of 5.0[51] and 50[52] µg/kg, respectively. These techniques have advantages but cannot quantify atrazine at ultratrace levels, use relatively large organic solvent volumes, and can produce relatively poor accuracy. Microwave assisted extraction coupled with solid-phase microextraction (MAE-SPME), as reported in Table 2.5, have also been used for extracting atrazine from soil, generating LOQs of 10,[53] and 5.0 µg/kg,[54] respectively, which cannot be considered ultratrace. Additionally, the reported accuracies were poor, the Shen et al.[54] method used a relatively large volume of methanol, and the Hernandez et al.[53] method produced poor precision. One of the preferred methods to extract atrazine from soil is solid phase extraction (SPE). A recent

study [55] utilized SPE to extract atrazine from soil, with other pesticides, producing the lowest LLOQ (1.0 µg/kg) of the methods discussed; however, the method was extraordinarily lengthy, requiring 16 hr of solvent extraction (i.e., 4 hr of shaking soil with pure water and 12 hr of shaking with methanol prior to SPE), required large solvent volumes (50 mL of methanol), produced relatively low accuracy, and was not able to achieve ultratrace LLOQs. Because each method discussed cannot be considered ultratrace and has additional disadvantages, a more sensitive, simple, and green method to detect ultratrace concentrations of atrazine was sought.

First described by Maslamani et al.,[45] ICECLES combines two techniques: stir bar sorptive extraction (SBSE) and freeze concentration (FC). ICECLES provides a simple and automated microextraction alternative that minimizes matrix interference and preconcentrates analytes. ICECLES has been used for extraction of green tea for food/flavor analysis, where ICECLES extracted over 300 compounds from green tea, 54 more than SBSE.[3] Additionally, two studies comparing ICECLES to SBSE and SPME for the extraction of over 300 pesticides, including atrazine, in deionized water and drinking water, ICECLES greatly outperformed the other extraction techniques. In purified water, ICECLES was able to detect ($S/N \geq 3$) 273 of the 313 compounds (87%) at 100 ng/L, versus 229 (73%) and 192 (61%) for SBSE and SPME, respectively.[56] In drinking water, ICECLES was able to detect 32 compounds at 0.1 ng/mL, versus 25 and 13 for SBSE and SPME, respectively.[57] ICECLES has also been used to successfully detect ultratrace concentrations of nitrosodipropylamine (LOD of 0.2 pg/mL), a difficult to analyze carcinogen, in drinking water.[4]

Since ICECLES has shown an ability to detect ultratrace concentrations of difficult to extract analytes while minimizing matrix effects, ICECLES was evaluated for extracting atrazine from soil. The goal of this study was to develop a reliable and sensitive extraction method for analysis of atrazine in soil using ICECLES. Soil samples from multiple locations in the U.S. were analyzed via this method to determine its utility in detecting atrazine at ultratrace concentrations.

2.2 Experimental

The following sections describe the materials and methods used for the analysis of atrazine from soil using ICECLES-HPLC-MS/MS.

2.2.1 Materials

Neat standards for both atrazine and its internal standard (atrazine-d_5) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol (LCMS grade), acetonitrile (LCMS grade), and formic acid (LCMS grade) were purchased from Fisher Scientific (Waltham, MA). The stir bars used for ICECLES were Gerstel Twister^{TM} stir bars (Lot #: 020120161117) with a 10 mm length and 0.5-mm film thickness. A MilliQ® Ultrapure water system (MilliporeSigma) was used to generate purified water.

2.2.2 Soil samples

Soil for method development was provided by the pesticide residue laboratory at SGS North America, Inc. in Brookings, South Dakota. The soil was collected from nearby field sites that had not recently used atrazine. Each sample's composition varied greatly (i.e., SD vs. CA soil) and their makeup and properties (i.e., % sand, % silt, pH, etc..) can be found in Table 2.4. Soil samples were air-dried, pulverized/homogenized, and sieved to ensure homogeneity. These prepared samples were pre-screened for

atrazine prior to using them as blank material for fortifications. Soil samples that produced undetectable levels of atrazine were used for method development and validation. Several potential blank samples (approximately 40% of those tested) were unusable soil for method development since they contained detectable amounts of atrazine. While enough blank soil was obtained as is for this study, we found that atrazine could be removed from soil by heating the soil samples to 500 °C for at least 12 hr. Since we were able to find enough untreated soil with undetectable levels of atrazine for method development, we did not use the heating procedure for this study since we were concerned that it may alter the soil matrix.

2.2.3 Standard preparation

A stock solution of atrazine was prepared by accurately weighing approximately 10 mg of the reference (neat) standard into a 10 mL volumetric flask and diluting with acetonitrile, producing a concentration of 1 mg/mL. Intermediate standard solutions were prepared in acetonitrile at 10 µg/mL and 100 ng/mL by one and two 1:100 serial dilutions, respectively. From these intermediate standard solutions, working solutions (0.010 to 10 ng/mL) were prepared in purified water. Similar serial dilutions in purified water were made for the internal standard (atrazine-d_5).

2.2.4 Fortification procedure

Blank soil (10 g) was placed into a 50 mL centrifuge tube and fortified at the desired concentration by adding 100 µL of the appropriate working standard. Next, internal standard (100 µL of 50 ng/mL) was added to each sample resulting in a final internal standard soil concentration of 500 ng/kg. Once the soil sample was spiked with atrazine and internal standard, the centrifuge tube was capped and the sample was lightly

shaken by hand for 30 s to mix. The sample was then left to stand on the benchtop for approximately 15 min prior to extraction.

2.2.5 Liquid chromatography—tandem mass spectrometry

Analysis of the ICECLES back-extract for atrazine was carried out using a Shimadzu Nexera XR HPLC (Tokyo, Japan), and a Sciex 6500+ MS/MS (Redwood City, CA). A Phenomenex Kinetex C18 column, 4.6 x 50 mm, 2.6 µm (Torrance, CA) with 0.1% formic acid in water (Mobile Phase A), and 0.1% formic acid in acetonitrile (Mobile Phase B) were used to separate atrazine. The HPLC gradient was initially 40% B, linearly increased to 95% B over 5.5 min, and held for 3 min, decreased back to 40% B over 0.1 min, and then held constant for 1.4 min (10-min total run time). The flow rate was 0.5 mL/min. The column and autosampler temperatures were 30 °C and 10 °C, respectively. An injection volume of 10 µL was used with 500 µL of an autosampler rinsing solution (acetonitrile:IPA:methanol (1:1:1)) used before and after aspiration. The MS/MS was operated at 500 °C, with an Ion Spray voltage of 5500 V, ion source gas 1 pressure of 70 psi, ion source gas 2 pressure of 70 psi, curtain gas pressure of 20 psi, and collision gas pressure of 10 psi. Various mass spectrometry parameters for atrazine are shown in Table 2.1.

Table 2.1 Mass spectrometry parameters.

Analyte	*m/z*	Declustering Potential (volts)	Entrance Potential (volts)	Collision Energy (volts)	Cell Exit Potential (volts)
Atrazine	216.1 → 173.9	80	10	23	16
Atrazine[1]	216.1 → 103.9	80	10	37	16
Atrazine-d_5	221.0 → 179.0	61	10	25	18

[1]Confirmatory ion

2.2.6 Extraction method

Soil (10 g of blank, fortified, or field sample) was weighed and placed into a 50-mL centrifuge tube. The samples were extracted with 20 mL of methanol:water (8:2, v:v) on a wrist-action shaker for 30 min at room temperature. After shaking, samples were centrifuged for 5 min at 1800 x g. Post-centrifugation, 10 mL of supernatant was transferred to a 15-mL centrifuge tube and the methanol was removed under steady nitrogen flow in a 40 °C water bath. Full methanol removal, under these conditions, lasted approximately 30 min. The samples were then reconstituted with 10 mL of purified water and then extracted with ICECLES.

2.2.7 ICECLES

The ICECLES apparatus used for this study was described by Maslamani et al.[45] ICECLES was performed by directionally freezing the sample solution from the bottom of the vial to the top as the solution was vigorously stirred. Freezing of the sample solution was accomplished with the aid of coolant flow (-5 °C) through a double-walled beaker. Stirring of the samples with a sorptive stir bar was initiated at 1200 rpm and subsequently reduced to 300 rpm once the height of the liquid solution was approximately 1 cm above the ice. Reducing the stir speed to 300 rpm was necessary to reduce “splashing” of the liquid sample as the stir bar neared the top of the sample. Once ICECLES was complete (i.e., the solvent was completely frozen), the stir bar was removed, placed into a 2-mL vial, and 500 µL of methanol was added to completely submerge the stir bar. The 2-mL vial was lightly shaken for approximately five seconds and left on the benchtop for approximately 10 minutes to back-extract atrazine. After back extraction was finished, the methanol was transferred to an HPLC vial for analysis.

2.3 Results and discussion

2.3.1 Soil method validation

The method was validated by generally following two SANCO guidelines: 12571/2013, the guideline for pesticide residue analysis in food and feed,[58] and 825/00, Rev. 1, the guidance document on residue analytical methods.[59]

The ability to differentiate atrazine from other interferents in the soil was determined by extraction and analysis of blank soil and soil fortified with atrazine. Selectivity was evaluated by comparing the signal of atrazine from the fortified soil to the signal produced by the blank soil at the retention time of atrazine (~3.5 min). The selectivity was further evaluated by calculating the resolution of the atrazine peak from the nearest peak which consistently produced detectable signals ($S/N \geq 3$).

Calibration curves were constructed for atrazine from 10 to 1000 ng/kg in soil alongside blank samples over multiple days (i.e., three separate calendar days within seven calendar days). Specifically, calibration standards, in triplicate, were prepared using the fortifying procedure discussed. The calibrators were then extracted and analyzed by the method presented here. The peak areas for both atrazine and atrazine-d_5 were calculated by integration from baseline to baseline in MultiQuant software. The average peak area signal ratios of atrazine to atrazine-d_5 were plotted as a function of concentration. The calibration curve was fit with both weighted ($1/x$ and $1/x^2$) and non-weighted least squares techniques and a $1/x$ weighting was chosen based on visual inspection of residuals and evaluation of the Percent Residual Accuracy [60] for multiple calibration curve ranges (i.e., numerous combinations of dynamic ranges and weighting factors were evaluated to find the most appropriate linear range).

Three QC standard concentrations were prepared in soil (50, 250, and 500 ng/kg as low, medium, and high QCs, respectively). Quality control standards were prepared in the same fashion as the calibrators. The QC standards were not included in the calibration curve but were used to estimate the accuracy and precision (%RSD) of the method by back-calculating the estimated concentration of the QCs based on the calibration curve and determining the %RSD as a measure of precision and the percent error at each QC concentration versus the true (nominal) QC concentration as a measure of accuracy. The QC standards were analyzed in quintuplicate on the same three days (within seven calendar days) that the calibration curves were constructed. The QCs were injected in parallel with the calibration standards. Intraassay accuracies and precisions were calculated from the QCs on each day's analysis and the interassay accuracies and precisions were determined by evaluation of the QCs over the entire three days. The limits of quantification (LOQs) (i.e., lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ)) were defined by investigating the above calibrators and determining the lowest concentration calibrator which satisfied the following criteria: (1) percent relative standard deviation (%RSD) of <10% (to measure precision) and (2) percent accuracy within ± 20% back-calculated from the nominal concentration of each calibration standard. The LOD was estimated by analyzing concentrations below the LLOQ and was defined as the lowest analyte concentration that consistently produced a S/N ratio of 3 with noise measured as peak to peak in the blank noise, over the retention time of atrazine. Moreover, symmetry of the chromatographic peak, as measured by peak asymmetry (A_s), was determined by dividing the front width by the back width at 10% peak height.

The matrix effect was determined by preparing calibration curves of atrazine spiked soil and water prepared as described and comparing the slopes. Non-equivalent slopes were interpreted as an indication of matrix effects. Method recovery of atrazine was determined from fortified soil and fortified solvent samples at two concentrations: 10 and 100 ng/kg (ng/L for solvent). Recoveries of atrazine were determined as a percentage by comparing peak areas obtained from the fortified soil with fortified solvent samples at the same concentrations. All recovery experiments were performed in triplicate.

2.3.2 Analysis of atrazine

The method presented here is a simple and sensitive technique for the ultratrace analysis of atrazine from soil consisting of a simple solid-liquid extraction using methanol and automated preconcentration and sample cleanup via the ICECLES technique and HPLC-MS/MS analysis. Although a sorptive stir bar is necessary for ICECLES, this technique precludes the need for single-use consumables, such as SPE cartridges, QuEChERS packets and/or dispersive-SPE materials. Methanol (16.5 mL), a relatively green solvent, is the only reagent necessary for the method. While some methods require less volume of organic solvent (methanol and/or acetonitrile) than the ICECLES technique, the method presented here produced an LLOQ 100-10,000x lower than these methods, as shown in Table 2.5.

Figure 2.1 shows a representative chromatogram of atrazine extracted from a spiked soil sample and analyzed via the ICECLES-HPLC-MS/MS method. The figure shows two atrazine MRM transitions, 216→174 (quantitation ion) and 216→104 (confirmatory ion), for spiked and non-spiked (i.e., “blank”) soil. The HPLC-MS/MS runtime was 10 min and atrazine eluted at 3.5 min. The peak shape for atrazine was sharp

and symmetrical with a peak asymmetry value of 1.1. While soil is an inherently difficult matrix to analyze, the high selectivity of the MS/MS measurement and the mitigation of matrix effects via ICECLES produced complete resolution from other components in the soil, as evident by the absence of co-eluting species in the blank over the elution time of atrazine.

2.3.3 LOD, LOQ, and Linear Range

The ICECLES-HPLC-MS/MS method produced excellent sensitivity for the analysis of atrazine from soil. The LOD was 5 ng/kg and the LLOQ was 10 ng/kg. The LLOQ of this method is orders of magnitude lower than any previously reported. Specifically, this method was able to generate an LLOQ 100x times lower than the next most sensitive method (SPE-LC-MS/MS) presented in Table 2.5, which used over 3x the organic solvent and required 4x the extraction time.

Calibration curves for the analysis of atrazine in soil were created in the range of 10 to 1000 ng/kg. Evaluation of multiple potential linear ranges (with a maximum concentration of 5000 ng/kg investigated) and weighting factors revealed that the linear range was 10 to 1000 ng/kg using 1/x weighting. The 5000 ng/kg calibrator fell out of linearity during evaluations and was removed from the dynamic range. Linearity for the analysis of atrazine in soil was excellent, with R^2 and PRA values for each calibration curve of ≥0.999 and 96 to 98%, respectively. Once the linear range was established, calibration curves were prepared on three separate days. The calibration curves were highly stable over the course of the three days, producing consistent slopes, intercepts, R^2s, and PRAs (Table 2.2).

Table 2.2 Regression data for inter-assay validation of atrazine in soil.

Day	Slope[1]	Intercept	R^2	PRA[2]
1	1.0070	0.0023	0.9999	97
2	0.9962	0.0013	0.9998	96
3	1.0036	0.0012	0.9999	98

[1]Utilizes ratio of atrazine and atrazine-d5.
[1]Percent Residual Accuracy

2.3.4 Accuracy, precision, matrix effect, and recovery

The ICECLES-HPLC-MS/MS method's accuracy and precision were evaluated at three concentrations (i.e., 50, 250, and 500 ng/kg) analyzed in quintuplicate over the course of three days (n = 15 for each concentration). Table 2.3 shows the intra- and interassay accuracy and precision of the method. The method generated satisfactory intraassay accuracy between 90 and 112% and excellent interassay accuracy between 95 – 109%. Intraassay precision was between 1.2 and 8.0% RSD and interassay precision was between 2.7 and 9.6% RSD. The precision of the method was very good considering the complexity of the soil matrix.

The comparison of solvent- and matrix-based calibration curves was used to evaluate potential matrix effects. A negligible matrix effect for analysis of atrazine in soil was found, with the ratio of slopes (i.e., $m_{\text{spiked soil}} / m_{\text{aqueous}}$) equal to 0.98. The recovery of atrazine from soil at 10 and 100 ng/kg was also excellent, ranging from 99 – 109, and 87 – 95%, respectively. The negligible matrix effect and excellent recovery can be attributed to ICECLES ability to efficiently extract the atrazine while leaving potential matrix interferents behind.

Table 2.3 Intra- and interassay accuracy and precision of QCs over 3 days of analysis.

Concentration of atrazine (ng/kg)	Intraassay accuracy (%)[a]	Interassay accuracy (%)[b]	Intraassay precision (%RSD)[a]	Interassay precision (%RSD)[b]
50	90 – 106	96	4.8 – 8.0	9.6
250	92 – 98	95	1.2 – 12	7.2
500	107 – 112	109	1.2 – 2.1	2.7

[a]Mean of the values for 1 day (n = 5 for each day); overall range for three separate days.
[b]Mean of the values over the three-day validation period (n = 15).

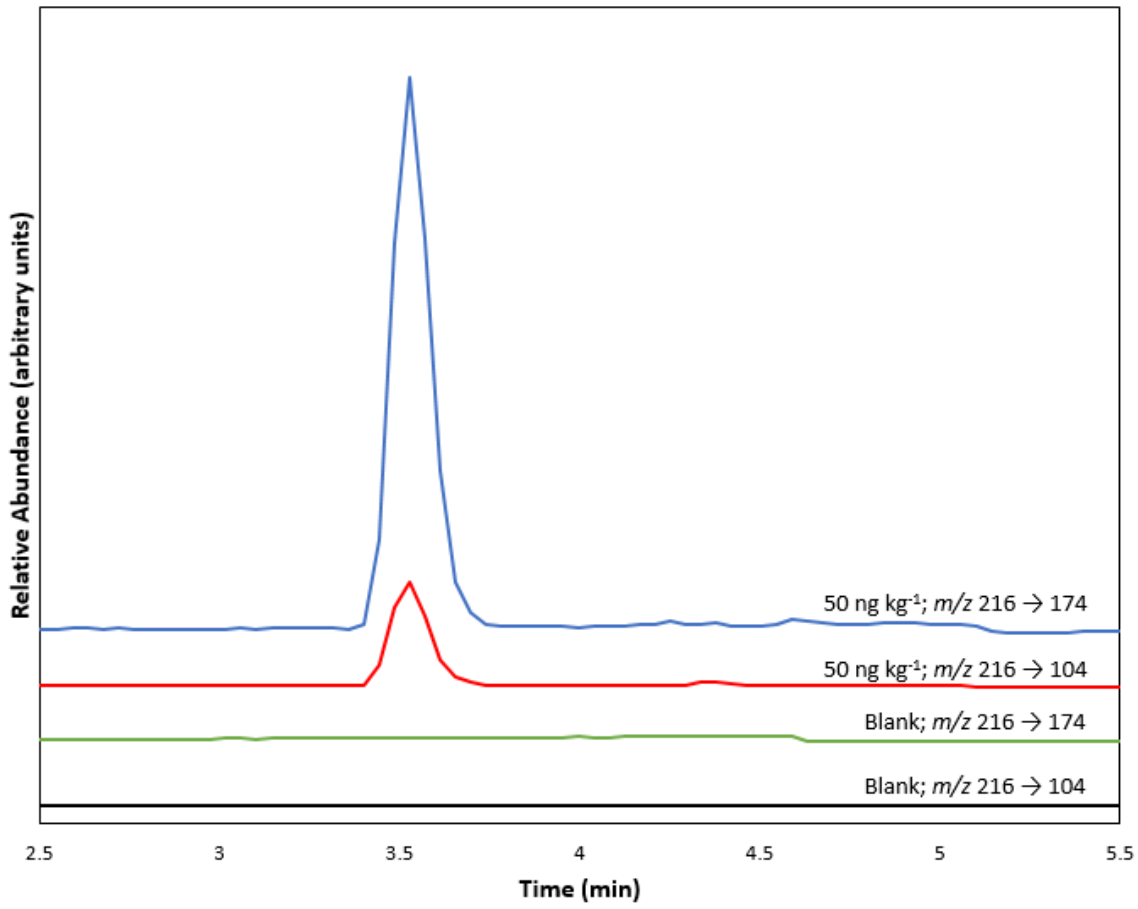


Figure 2.1 Chromatogram of a blank (unfortified) soil sample and a soil sample fortified at 50 ng/kg for atrazine.

2.3.6 Application of the method

In order to evaluate this newly validated method, seven field soil samples from locations across the U.S. were analyzed: two samples were from the central valley area of California, two from southern Georgia, and three from eastern South Dakota. These soil types offered a diverse group of samples with inherently different compositions and atrazine concentrations (listed in Table 2.4). Figure 2.2 shows representative chromatograms of atrazine extracted from soil originating from the three states. The highest concentrations of atrazine were detected in the South Dakota soil, averaging 1526 ng/kg. Atrazine was also detected in the soil from Georgia, with an average concentration of 30 ng/kg. Atrazine was not detected in either of the California soil samples. Relative atrazine concentrations found in these soils coincide with the geographical use of atrazine across the U.S., as atrazine is used significantly more in the Midwest on crops such as corn, than in the South and the West. For example, in 2017, South Dakota, Georgia, and California applied 860428, 313963, and 6828 kg of atrazine, respectively to corn crops.[61]

Table 2.4 Field soil sample composition and atrazine concentration.

Sample	Atrazine (ng/kg)	% Sand	% Silt	% Clay	pH	% Organic Matter
South Dakota #1	1121	35	40	25	6.0	3.0
South Dakota #2	549	35	38	27	6.1	3.0
South Dakota #3	2907	35	40	25	6.3	2.9
Georgia #1	31	86	5	9	5.9	0.55
Georgia #2	29	84	6	10	6.0	0.61
California #1	ND[a]	57	23	20	6.2	2.6
California #2	ND	56	22	22	6.1	2.7

[a]ND = Not detected

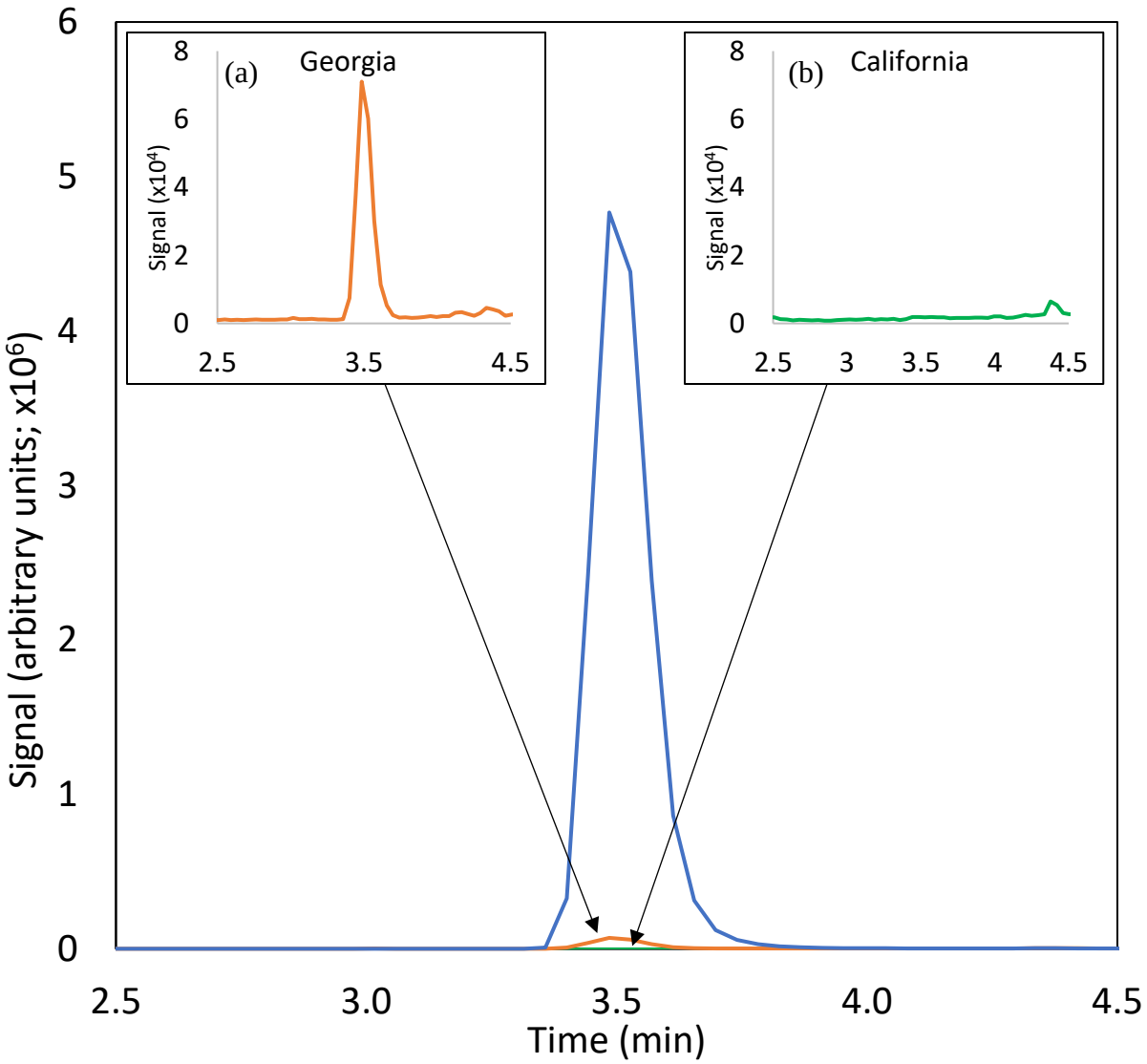


Figure 2.2 Chromatograms from ICECLES-HPLC-MS/MS analysis of South Dakota, Georgia (inset a), and California (inset b) soil for atrazine.

2.3.7 Method comparison

Table 2.5 compares parameters of selected techniques for atrazine analysis from soil, including LLOQs, accuracies, organic solvent volumes used, and extraction times for the ICECLES-HPLC/MS/MS method and the others mentioned previously. ICECLES generated an LLOQ 100x lower than the next most-sensitive technique listed, SPE-LC-MS/MS, and the best accuracy (96%) of all the methods listed. While this work required

approximately 4 hr to perform (i.e., the second longest of the methods presented), it produced much lower LLOQs (500-10,000x) than the methods with shorter sample preparation times. Furthermore, the method producing an LLOQ closest to the ICECLES-HPLC-MS/MS technique (i.e., the SPE-LC-MS/MS method) required 16 hr for the extraction and was 100x less sensitive.

2.3.8 Conclusion

As governments and regulatory bodies continue to require lower limits of detection for pesticides in foodstuffs, the ability to detect ultratrace concentrations of these compounds in the soil that grows the food is becoming increasingly important. The ICECLES-HPLC-MS/MS method presented here was able to generate an LLOQ for atrazine in soil of 10 ng/kg and quantify atrazine concentrations in field soil samples which accurately reflect the amount of pesticide being applied in different U.S. geographies. This ICECLES-based extraction technique provided an accurate and precise analysis of atrazine in soil at ultratrace levels. Because this method proved excellent for atrazine ultratrace analysis from soil, more analytes should be evaluated to better demonstrate the robustness of ICECLES.

Table 2.5 Comparison of extraction and analysis techniques for atrazine from soil.

Sample Preparation Technique[a]	Analysis Technique[b]	LLOQ (µg/kg)[c]	Accuracy (%)[d]	Precision (%RSD)[d]	Extraction time (hr)[e]	Organic Solvent[f]	Reference
USE	HPLC-DAD	5	83 (n = 15)	4.3 (n = 15)	2	5 mL of methanol	Wu et al., 2010 [#9]
USE-SPME	GC-ECD	100	53[g] (n = 12)	15 (n = 12)	1	5 mL of methanol	Bouaid et al., 2001 [#8]
QuEChERS	LC-MS/MS	50	86 (n = 5)	6.0 (n = 5)	2	25 mL of acetonitrile	Geronimo et al., 2015 [#11]
QuEChERS	GC-MS/MS	5	111 (n = 5)	16 (n = 5)	2	10 mL of acetonitrile and 1 mL of ethyl acetate	Yu et al., 2016 [#10]
MAE-SPME	GC-MS	10	77 (n = 3)	4.8 (n = 3)	1	25 mL of methanol	Shen et al., 2003 [#13]
MAE-SPME	GC-MS	5	67 (n = 6)	20 (n = 6)	1	5 mL of methanol	Hernandez et al., 2000 [#12]
SPE	LC-MS/MS	1	85 (n = 5)	7.0 (n = 5)	16	50 mL of methanol	Colazzo et al., 2018 [#14]
ICECLES	LC-MS/MS	0.01	96 (n = 15)	9.6 (n = 15)	4	16.5 mL of methanol	This work

[a] USE = Ultrasonic solvent extraction; SPME = Solid-phase microextraction; QuEChERS = Quick, Easy, Cheap, Effective, Rugged, and Safe; SPE = Solid-phase extraction; ICECLES = Ice concentration linked with extractive stirrer.

[b] HPLC = High Performance Liquid Chromatography; DAD = Diode Array Detector; GC = Gas chromatography; ECD = Electron Capture Detector; LC = Liquid Chromatography;
MS = Mass Spectrometry; MS/MS = Tandem Mass Spectrometry

[c] LLOQ = Lower limit of quantification.

[d] Accuracy and precision measured at lowest quantifiable concentration tested.

[e] Extraction time (hr) represents total time for sample preparation and extraction (i.e., extraction of the soil and subsequent preparation of the soil extract).

[f] This represents the total organic volume and identity of solvent (methanol, acetonitrile, ethyl acetate, acetone) needed for each sample.

[g] Represents average for the four soil types tested. The values had accuracies of 62, 47, 43, and 60, respectively.

Chapter 3. Comparison of the Extraction Efficiency of ICECLES, SBSE, SPME, LLE, and SPE for Pesticides from Pure and Drinking Water

3.1 Introduction

The trace analysis of pesticides in drinking water is paramount to human health, as these compounds inevitably find their way into our water sources, where they are unintentionally being consumed. Analytical extraction methods are needed to detect pesticides in drinking water, and ICECLES was determined to be an excellent candidate for their detection.

While ICECLES has shown promise, it had not been compared directly to other sample preparation techniques, besides SBSE, for a variety of compounds before this project. First, atrazine was used a probe analyte to determine the extraction efficiency of ICECLES, SBSE, SPME, LLE, and SPE in pure water and analysis via HPLC-MS/MS.

Additionally, a comparison of five analytes (furfural, 2-methyl pyrazine, 1-pentanol, indole, and atrazine) using ICECLES, SBSE, and SPME, was performed in pure water using TD-GC-MS.

Next, two separate experiments, each containing large suites of pesticides, N = 60, and 313, respectively, in purified water and drinking water were prepared via ICECLES, SBSE, and SPME and the extraction efficiency of each technique was compared.

The comparison of these sample preparation techniques was carried out on 60 different pesticides with analysis via TD-GC-MS and LC-MS/MS. ICECLES produced 2x and 7x greater TD-GC-MS signals than SBSE and SPME, respectively.

For the analysis of 313 pesticides, ICECLES produced up to 10x the signals for SBSE and up to 120x the signals for SPME.

3.2 Experimental

The following sections describe the materials and methods used for the comparative analysis of all compounds via ICECLES, SBSE, and SPME.

3.2.1 Materials

For ICECLES and SBSE, Gerstel Twister™ stir bars (Lot #: 020120161117) with 10 mm length, 0.5-mm film thickness (equating to a PDMS volume of 24 µL)[43, 62] were used. For SPME, 100-µm film thickness fibers were used (Supelco, Lot #: P333880), which equates to a PDMS volume of 0.612 µL. [63, 64] Reference standards for atrazine, furfural, 2-methylpyrazine, 1-pentanol, and indole were purchased from Sigma-Aldrich (St. Louis, MO). The larger suite of pesticides (N = 60 and N= 313) were purchased as pre-made, mixed standards in acetonitrile from AccuStandard (New Haven, CT). Acetonitrile (LCMS grade), methanol (LCMS grade), dichloromethane, acetic acid, hydrochloric acid, and formic acid were purchased from Fisher Scientific (Waltham, MA). ENVI-carb SPE cartridges (3 mL, 250 mg sorbent) were obtained from Supelco/Sigma-Aldrich (St. Louis, MO).

3.2.2 Progressive concentration of atrazine during ICECLES

To evaluate the extraction efficiency of ICECLES as the sample is freeze concentrated, an aqueous atrazine sample was prepared stepwise via ICECLES, with analysis of the stir bar at various stages of FC. Four samples, each containing 10 mL of purified water, were fortified with atrazine at 2.16 ng/mL (10 nM) and added to a 10-mL vial. The sample “height” was measured from the bottom of the vial to the meniscus. The vials were marked at 25, 50, 75, and 100% of the full sample height. The vial marked at 100% (full height) represented a fully frozen sample. The four samples were placed into

the ICECLES apparatus at the same time and under the same conditions: 1200 rpm stir speed and -5 °C. Once the ice reached the mark in each vial, the vial was removed from the ICECLES apparatus and the stir bar was removed and analyzed for atrazine via TD-GC-MS.

3.2.3 Sample preparation of aqueous atrazine

The method of Queiroz et al.[65] was adopted for LLE sample preparation, in which three portions (50 mL each) of dichloromethane were used to partition atrazine from 500 mL of water at pH 2.5. After partitioning, the aqueous portion was discarded and the dichloromethane (150 mL) was evaporated to dryness. Finally, the residue was reconstituted with 1 mL of methanol:water (6:4, v:v) and filtered before injection. In total, the extraction took approximately three hours and required constant attention during the LLE step.

The SPE method for this study was adopted from Trajkovska et al.[66], where an ENVI-carb cartridge was used to extract atrazine. After conditioning the cartridge, 1000 mL of sample was passed through the SPE cartridge. The atrazine was eluted first with 1.5 mL of methanol and then with a mixture of dichloromethane and methanol (8:2, v:v). Next, the eluate was evaporated to dryness, reconstituted with 1.5 mL of methanol and filtered. In total, the extraction took approximately four hours. Sample addition to the SPE cartridge was the longest step (i.e., 3 hr) and required constant attention.

For the SBSE extraction, PDMS stir bars were placed into glass scintillation vials containing 10 mL of sample (blank or fortified). The stir bars were stirred at ≈1200 rpm for three hours at room temperature (i.e., the longer than normal SBSE extraction time was used to allow a more direct comparison to ICECLES). At that point, the stir bars

were removed, placed into a centrifuge tube, and back extracted with 500 µL of methanol.

SPME analysis, specifically direct immersion, of pesticides have been performed previously in other studies.[67, 68] For this study, the PDMS fiber was immersed for three hours in 10 mL of water fortified with atrazine (i.e., as with SBSE, the extraction time was extended beyond typical SPME extractions to allow direct comparison to ICECLES). The fiber was then back-extracted with 500 µL (i.e., to mimic ICECLES and SBSE) or 55 µL of methanol (i.e., the smallest volume of back-extraction solvent necessary to completely submerge the SPME fiber in a 150 µL HPLC vial insert). Each of the atrazine extracts were analyzed via LC-MS/MS via the method described below.

3.2.4 Extended comparison of ICECLES and SBSE for atrazine extraction

Due to their similarities, a further comparison of SBSE and ICECLES was carried out. SBSE was performed at room temperature (≈20 °C) and ICECLES was performed at -5 °C. For each technique, 5, 25 and 50 pg/mL atrazine spikes were extracted in triplicate. All stir bars were back-extracted with 500 µL of methanol with vortexing. All samples (i.e., 18 total) were analyzed on the same day via LC-MS/MS against a set of calibration standards.

3.2.5 Analysis of compounds via TD-GC-MS

To compare the performance of ICECLES, SBSE, and SPME for TD-GC-MS, each technique was used as described above to prepare atrazine and four additional compounds: (furfural (log K_{ow} = 0.41), 2-methylpyrazine (log K_{ow} = 0.49), 1-pentanol (log K_{ow} = 1.35), and indole (log K_{ow} = 2.05)[69,70]. These compounds were purposefully selected to evaluate compounds with a range of log K_{ow}s (0.41 to 2.7). Stock standards

for each compound were made in water, except for atrazine, which was prepared in acetonitrile to ensure solubility. Next, a mixed standard was made containing all five compounds in water with serial dilutions, as necessary, made with deionized water. Samples (10 mL) were spiked in triplicate at three different concentrations: 1, 10 and 100 nM with the mixed standard. Additionally, three blank (unspiked) water samples were analyzed. As mentioned previously, typical SBSE and SPME extraction times are less than that of ICECLES, however, to eliminate bias and maximize experimental consistency, all samples were extracted on the same day under the same conditions, including extraction times. While shorter extraction times are more typical for SBSE and SPME, it has been found that recoveries of these techniques increase with longer extraction times[62, 71].

3.2.6 Comparison of 60 pesticides in pure and drinking water

Comparison of the extraction efficiencies of ICECLES, SBSE, and SPME for 60 pesticides was performed in purified water and drinking water. Three different aqueous concentrations (0.1, 1.0, and 10 ng/mL) of these pesticides were compared. For the drinking water analysis, per EPA guidance[72], water was collected from a drinking water tap after ample purging of the line occurred (five minutes). Approximately 1 L of water was collected in a new, clean glass bottle. From this 1-L sample, 10 mL aliquots were taken and placed into 15 mL centrifuge tubes and then fortified with all sixty compounds. Each analysis was performed in triplicate. The same extraction parameters as described above (Section 3.2.2) for the extraction of atrazine via ICECLES, SBSE, and SPME were used in this experiment. SBSE stir bars and SPME fibers were back extracted as described above (Section 3.2.2).

3.2.7 Comparison of 313 pesticides in pure water

A comparison experiment was conducted on 313 pesticides in pure water, in order to determine which technique, ICECLES, SBSE, or SPME produces the greatest signal for a wide variety of pesticide classes.

3.2.8 LC-MS/MS Analysis

Solvents used for back-extraction of ICECLES, SBSE, SPME, and those used for reconstitution of SPE and LLE were analyzed with a Shimadzu Nexera XR HPLC (Tokyo, Japan). For atrazine analysis, a Phenomenex Kinetex C18 column, 4.6 x 50 mm, 2.6 µm (Torrance, CA), and Sciex 6500+ MS/MS (Redwood City, CA). Mobile Phase A was 0.1% formic acid in water and Mobile Phase B was 0.1% formic acid in acetonitrile. The HPLC gradient was initially 40% B, linearly increased to 95% B over 5.5 min, and held for three min, decreased back to 40% B over 0.1 min, and then held constant for 1.4 min (10-min total run time). The flow rate was 0.5 mL/min. The column and autosampler temperatures were 30 °C and 10 °C, respectively. An injection volume of 10 µL was used with 500 µL of an autosampler rinsing solution (acetonitrile:IPA:methanol (1:1:1)) used before and after aspiration. The MS/MS was operated at 500 °C, with an Ion Spray voltage of 5500 V, ion source gas 1 of 70 psi, ion source gas 2 of 70 psi, curtain gas at 20 psi, and collision gas at 10 psi. The primary ion transition used for the quantitation of atrazine was *m/z* 216.1 → 173.9, with *m/z* 216.1 → 103.9 as the confirmatory transition.

The suite of extracted pesticides was separated an Eclipse XDB-C18 3.5 µm, 2.1 x 150 mm HPLC column (Agilent, Part #: 930990-902). Mobile Phase A was 5 mM ammonium formate with 0.1% formic acid in water and Mobile Phase B was 5 mM ammonium formate with 0.1% formic acid in acetonitrile. The HPLC gradient was

initially 30% B, linearly increased to 98% B over 20 min, and held for five min, decreased to 30% B over one min, and then held constant for four min (30-min total run time). The flow rate was 0.3 mL/minute. The column and autosampler temperatures were 25 °C and 10 °C, respectively. The MS/MS was operated at 400 °C, curtain gas at 35 psi, and collision gas at 12 psi. The retention times, primary ion transitions used for quantitation, and the mass spectrometer parameters of these sixty compounds can be found in Table 3.1.

LC-MS/MS analysis for all compounds was performed in positive polarity mode with an entrance potential of 10 V.

3.2.9 TD-GC-MS Analysis

A Gerstel MPS was used in tandem with an Agilent 7890A GC with 5975C Inert Mass Spectrometer with electron ionization and a DB-5MS column (30 m x 0.25 mm, 0.25 µm) for analysis. The TD initial temperature was 40 °C, held for 1 minute, then ramped to 250 °C and held for 1.5 min. The desorption mode was splitless, with a standby cooling temperature of 50 °C. A coolant injection system (CIS) utilizing liquid nitrogen was used at -100 °C. The GC oven temperature was held constant at 35 °C for 1 minute and linearly increased (30 °C per minute) to 280 °C, then held for 3 minutes, before post-run equilibration. The total run time was 11.1 minutes. Helium was utilized as the carrier gas and liquid nitrogen was utilized to mitigate gas expansion in the inlet and to better focus the desorbed analytes onto the GC column. Table 3.2 lists the log K_{ow}s, quantification fragment ion m/zs, and retention times for each compound.

Table 0.1 Mass spectrometer parameters for sixty compounds

Compound	*m/z*	Retention Time	DP[1]	CE[2]	CXP[3]
Aldicarb	208 → 89	7.8	50	25	10
Aldicarb-sulfoxide	207 →132	1.9	61	9	14
Aldicarb-sulfone	240 → 86	2.2	28	28	4
Aminocarb	209 → 152	1.7	40	25	6
Amitraz	212 → 170	9.7	40	18	10
Acibenzolar-S-Methyl	211 → 136	14.4	26	31	8
Bendiocarb	224 → 167	9.9	25	25	10
Butylate	218 → 57	19.0	85	30	10
Carbaryl	202 → 145	10.8	51	13	8
Carbendazim	192 → 160	3.4	61	25	10
Carbofuran	222 → 165	1.6	65	17	10
Chlorantraniliprole	484 → 453	13.5	61	21	12
Chlorodimeform	197 → 46	3.9	50	20	10
Chlorbufam	241 → 172	14.3	50	25	10
Chlorpropham	214 → 154	15.1	25	10	10
Cymiazole	219 → 171	5.8	50	30	10
Cycloate	216 → 154	18.4	45	20	10
Desmedipham	318 → 182	13.3	50	20	10
Diallate	270 → 86	18.7	45	25	10
Dimepiperate	264 → 146	18.5	20	15	10
Dioxacarb	301 → 168	13.6	76	12	10
Diphenamid	240 → 134	13.0	70	20	10
EPTC	190 → 128	16.7	65	20	10
Ethiofencarb	226 → 107	11.2	50	25	10
Etobenzanid	340 → 179	17.4	60	20	10
Fenfuram	202 → 109	11.1	40	20	10
Fenoxycarb	302 → 88	16.6	80	35	14
Fenthiocarb	254 → 72	16.7	80	35	10
Fenoxanil	329 → 86	16.4	60	25	10
Formetanate HCl	222 → 165	1.6	40	25	10
Fuberidazole	185 → 157	4.9	50	35	10
Furathiocarb	383 → 195	19.2	65	30	4
Iprovalicarb	321 → 119	15.6	66	30	10
Isopropalin	310 → 226	23.6	120	20	10
Isocarbamid	186 → 87	6.7	60	20	10
Mepronil	270 → 119	15.1	90	35	12
Methiocarb	226 → 121	14.3	70	30	10
Methomyl	163 → 106	2.9	61	15	12

Table 0.2 (continued) Mass spectrometer parameters for sixty compounds

Compound	*m/z*	Retention Time	DP[1]	CE[2]	CXP[3]
Metolcarb	166 → 109	8.7	80	20	10
Napropamide	272 → 129	15.9	50	25	10
Naproanilide	292 → 171	16.4	40	15	10
Oxamyl	237 → 72	2.2	50	25	10
Oxamyl oxime	163 → 72	1.9	40	15	10
Oryzalin	347 → 288	16.1	40	20	10
Phenmedipham	301 → 136	13.6	70	30	12
Pirimicarb	239 → 72	7.8	50	35	10
Promecarb	208 → 109	14.6	40	25	10
Propamocarb HCl	189 → 102	1.9	45	25	10
Propanil	218 → 162	14.3	30	25	10
Propham	180 → 138	12.0	40	15	10
Thiabendazole	202 → 131	4.4	85	45	10
Thiodicarb	355 → 88	11.5	50	25	10
Triallate	304 → 143	20.2	65	35	10
Trichlamide	340 → 121	17.3	100	43	14
2,3,5-Trimethacarb	194 → 137	12.0	35	15	8
3,4,5-Trimethacarb	194 → 137	12.0	30	15	10
Vernolate	204 → 128	18.1	30	15	10
XMC	180 → 123	10.9	25	20	10
Xylylcarb	180 → 123	11.1	140	46	17
Zoxamide	336 → 187	17.5	140	46	17

[1]DP = Declustering potential (volts).
[2]CE = Collison cell energy (volts).
[3]CXP = Collision cell exit potential (volts).

Table 0.3 Properties of compounds used for comparison of SPME, SBSE, and ICECLES with analysis via TD-GC-MS.

Compound	Log K_{ow}	Quantification *m/z*	Retention Time (min)
Furfural	0.41	96	3.78
2-methylpyrazine	0.49	94	3.70
1-pentanol	1.35	55	3.3
Indole	2.05	117	6.4
Atrazine	2.7	200	8.3

3.3 Results and discussion

3.3.1 Extraction efficiency of atrazine during progressive freezing

The extraction efficiency of ICECLES for atrazine during progressive freezing was initially evaluated to determine the extraction profile as the aqueous sample freezes. The chromatograms are compared in Figure 3.1 along with the peak area comparison, inset, as a percentage of the maximum area (i.e., the 100% frozen sample). The area counts of atrazine extracted from water were 13%, 22%, and 50% of the area counts of the fully frozen sample at 25%, 50%, and 75% freezing of the sample, respectively. This geometric increase in extraction efficiency shows the importance of the freezing conditions in the later stages of ICECLES and that complete freezing of the sample is important to ensure the highest extraction efficiency. Currently, the stir speed of the sorptive stir bar is reduced to minimize "splashing," but it is likely that even better extraction efficiency could be achieved through detailed control of freezing, especially during the final stages. An apparatus is being developed in our lab to allow more highly controlled ICECLES and will be reported in the near future.

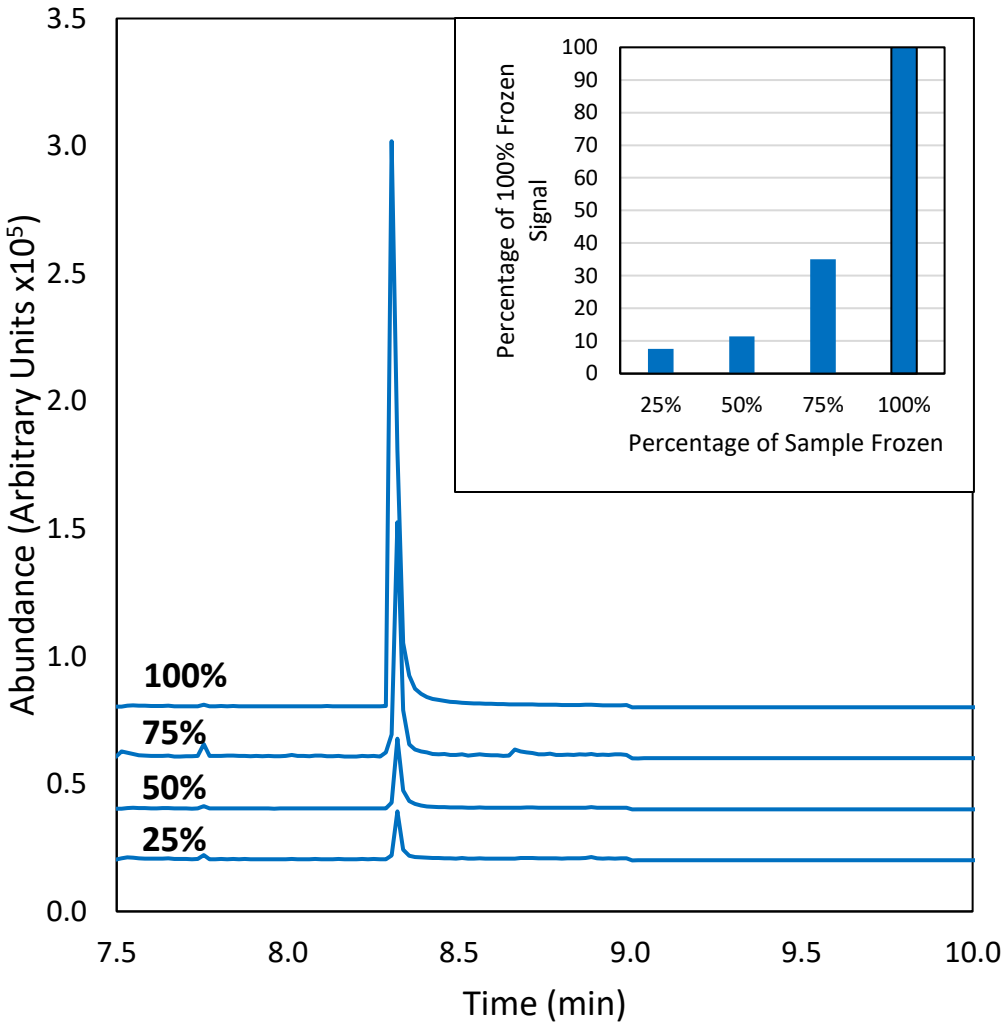


Figure 0.1 Chromatograms of aqueous atrazine samples at various stages of freezing during ICECLES sample preparation. Inset: Extraction efficiency of ICECLES normalized to the signal of the 100% frozen sample.

3.3.2 Results for atrazine comparison in pure water

Each sample preparation technique of interest was used to extract atrazine from water. Table 3.3 lists some requirements of each of these techniques and how they compare to one another with respect to time/labor, sample size used, recovery, and automation. In addition to the comparisons made in Table 3.3, it should be noted that

both the SPE and LLE methods required specialized glassware/equipment (separatory funnels, evaporators) and additionally, SPE requires non-reusable consumables (cartridges). Moreover, due to their larger sample size requirements, LLE and SPE also generate a relatively large amount of waste per sample and require constant attention. ICECLES also requires a specialized setup; however, the apparatus, once installed, does not require constant cleaning (i.e. LLE glassware), or constant replacement of consumables (i.e., SPE cartridges). It should be reiterated that sample preparation times for SBSE and SPME are typically approximately one hour. For this study, the SBSE and SPME extractions were purposefully carried out for longer periods of time to allow equilibrium to be reached and to create a direct comparison to the ICECLES extraction. It should be noted that previous studies using SPME for atrazine have produced equilibrium times varying from 30 minutes[49] to four hours[53].

A SPME extraction profile for atrazine was performed in pure water to determine equilibrium. Each water sample (10 mL) was fortified with atrazine at 100 pg/mL, with triplicate samples at the following time intervals: 30, 60, 90, 120, and 180 minutes. SPME fibers were directly immersed in the water samples with a small stir bar in the bottom of each vial. The vials were stirred at 1200 rpm. After two hours, the remaining samples (180 minute interval) had their stirring reduced to 300 rpm to match the comparison experiments in this study. Additionally, in order to match the comparison experiments reported here, the fibers were removed from their respective vials and back-extracted with methanol (55 µL). Samples were then analyzed for atrazine via HPLC-MS/MS with the same parameters reported in Section 2.8. Figure 3.2 depicts the mean of the triplicate samples at each time interval.

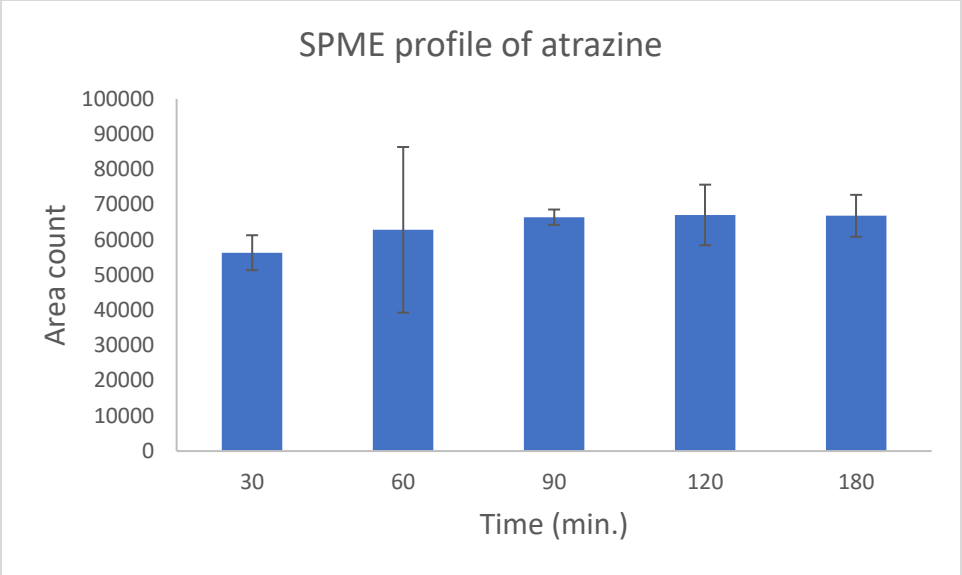


Figure 0.2 SPME extraction profile for atrazine in pure water.

A direct comparison of these techniques (evaluated in triplicate) was further assured by utilizing the same sorbent material (PDMS), the same volume of sample (10 mL), and the same stirring speed (1200 rpm).

Table 0.4 Comparison of techniques used to extract atrazine (5 pg/mL) from water

Technique	Sample Size (mL)	Automated	Signal Relative to SBSE	Recovery (%)
SPE	1000	No	2.3	3.2
LLE	500	No	2.2	6.4
SBSE	10	Yes	1.0	14
SPME[1]	10	Yes	$2.9x10^{-3}$	ND[2]
SPME[3]	10	Yes	$6.4x10^{-3}$	ND[2]
ICECLES	10	Yes	4.9	70

[1]Back-extracted with 500 µL
[2]ND = not detected
[3]Back-extracted with 55 µL

Comparing the extraction efficiency of each sample preparation technique demonstrated the ability of ICECLES to produce greater signal intensities and percent recoveries of atrazine from water. Figure 3.3 presents the comparison of SPE, LLE, SPME, SBSE, and ICECLES for the extraction of atrazine from water. The actual

recovery, reported as a percentage, was calculated by dividing the recovered concentration by the fortified concentration. At aqueous atrazine concentrations of 5 pg/mL, an average of ≈70% recovery was achieved using ICECLES with signal intensities of ≥2.5x SPE and LLE. SBSE produced recoveries of ≈14%, while LLE and SPE achieved recoveries of 6.4 and 3.2%, respectively. In fact, at this atrazine concentration, SPME did not produce signals above the noise (Figure 3.3 inset). Although the SPE and LLE produced the second highest signal intensities, very large sample volumes (1000 mL for SPE and 500 mL for LLE) were necessary to produce these signals. Therefore, SPE and LLE actually produced much lower recoveries than either SBSE or ICECLES. Further evaluation of SPME at higher concentrations (using 55 µL for back-extraction) revealed that an atrazine concentration of 50 pg/mL (10x more concentrated) was necessary to produce detectable atrazine signals. While an analyst might consider the signal produced by SBSE for atrazine as close enough to ICECLES to warrant its use (i.e., to avoid the ICECLES equipment requirements and longer sample preparation times), the signal enhancement of ICECLES compared to SBSE has been shown by Maslamani et al.[2] to quickly increase as the log K_{ow} of the analyte decreases.

The concentration factor of LLE, SPE, SBSE, SPME (0.5 mL back-extraction), SPME (0.055 mL back-extraction), and ICECLES are 0.002, 0.0015, 0.05, 0.05, 0.0055, and 0.05, respectively. Based on these enrichment factors, LLE and SPE should have produced better signals; however, these references produced LOQs of 100 and 2000 pg/mL, respectively, based on their methods. It's conceivable that these methods are unable to detect atrazine at 5 pg/mL. With respect to SPME, the lower volume of

stationary phase of the fiber (compared to SBSE and ICECLES stir bars) is certainly a large contributor to the lack of detection of atrazine at 5 pg/mL.

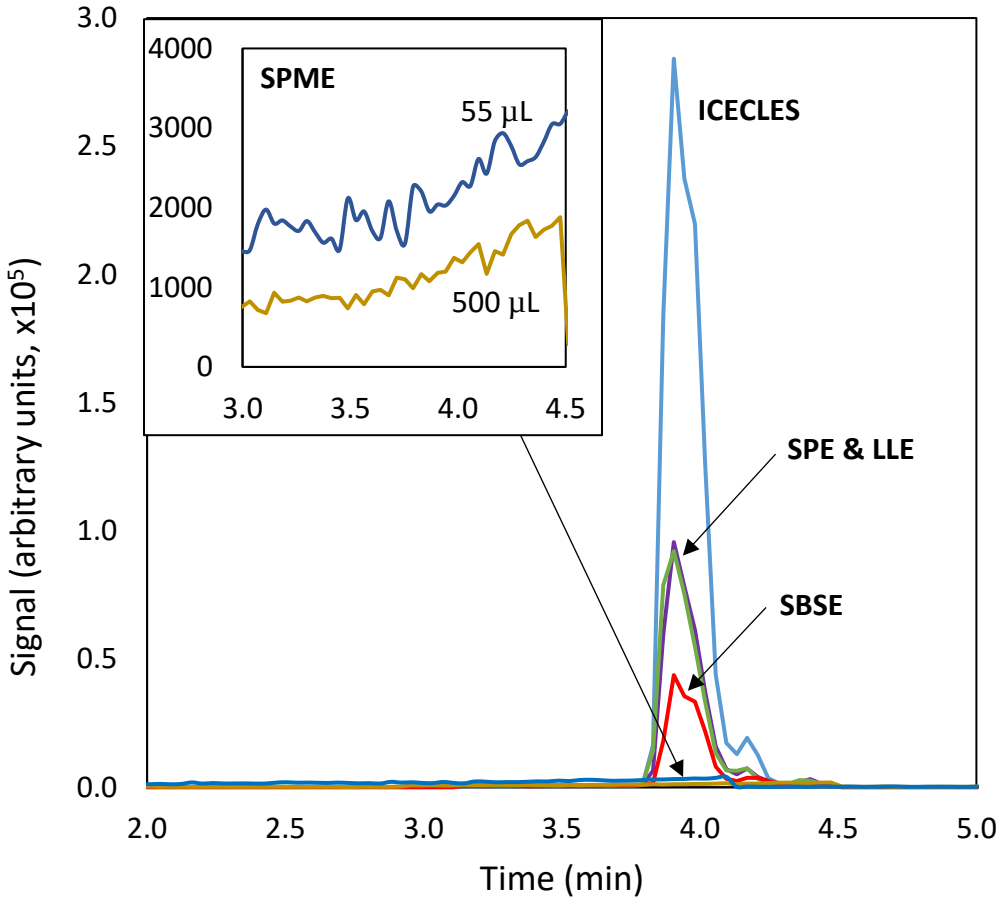
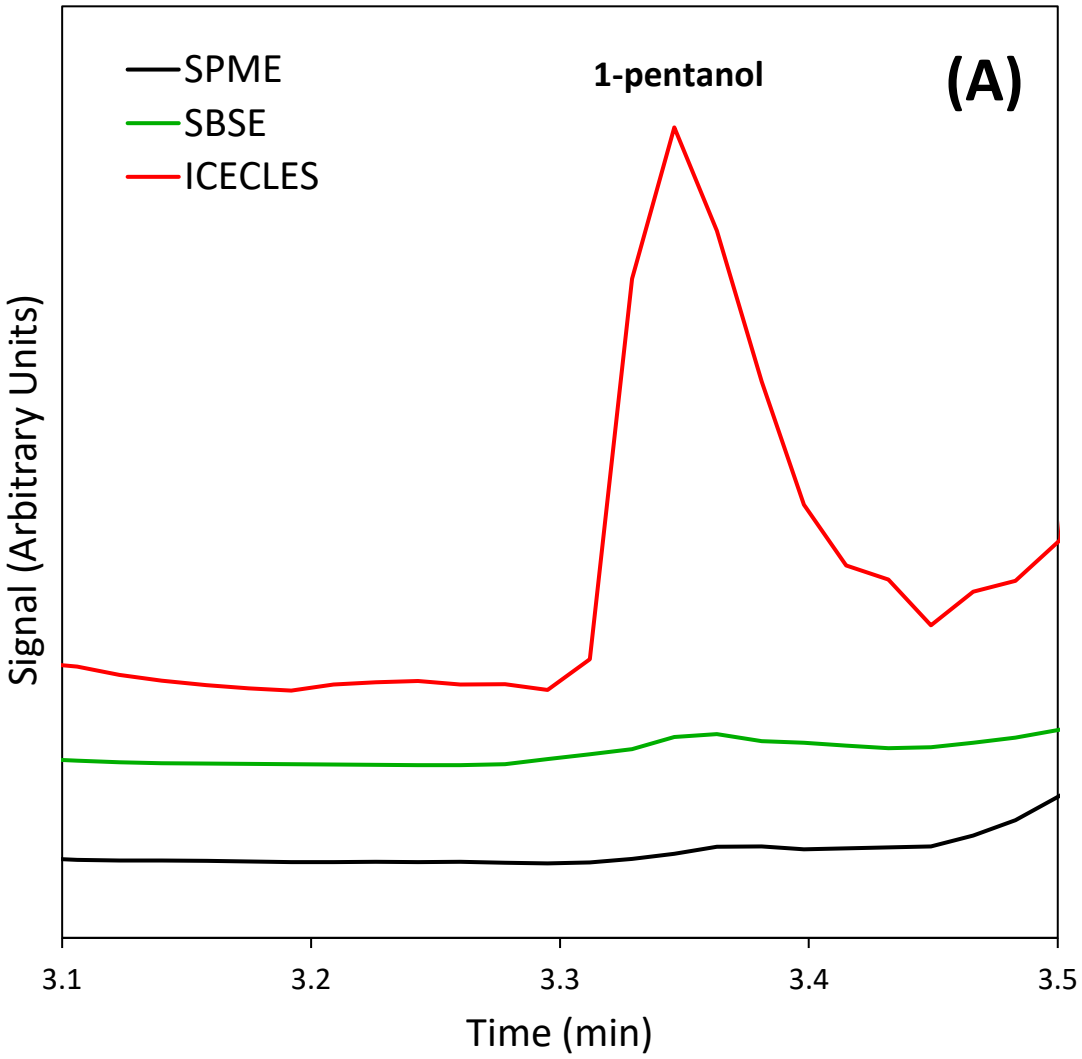


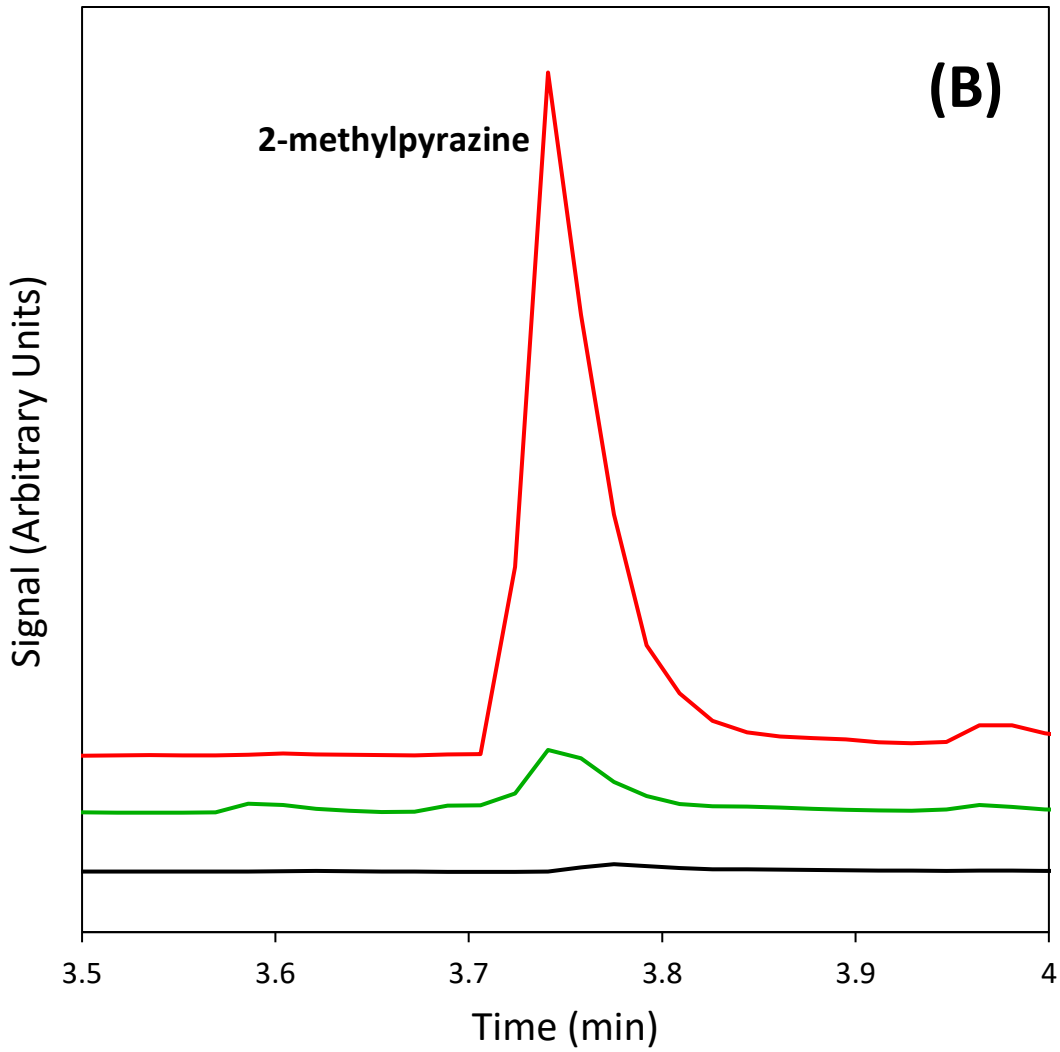
Figure 0.3 Signal intensity of atrazine (5 pg/mL) prepared via the described sample preparation techniques. ICECLES produced higher signals than SPE, LLE, SBSE, and SPME. Inset: SPME 500 µL back-extraction (gold), and SPME 55 µL back-extraction (blue).

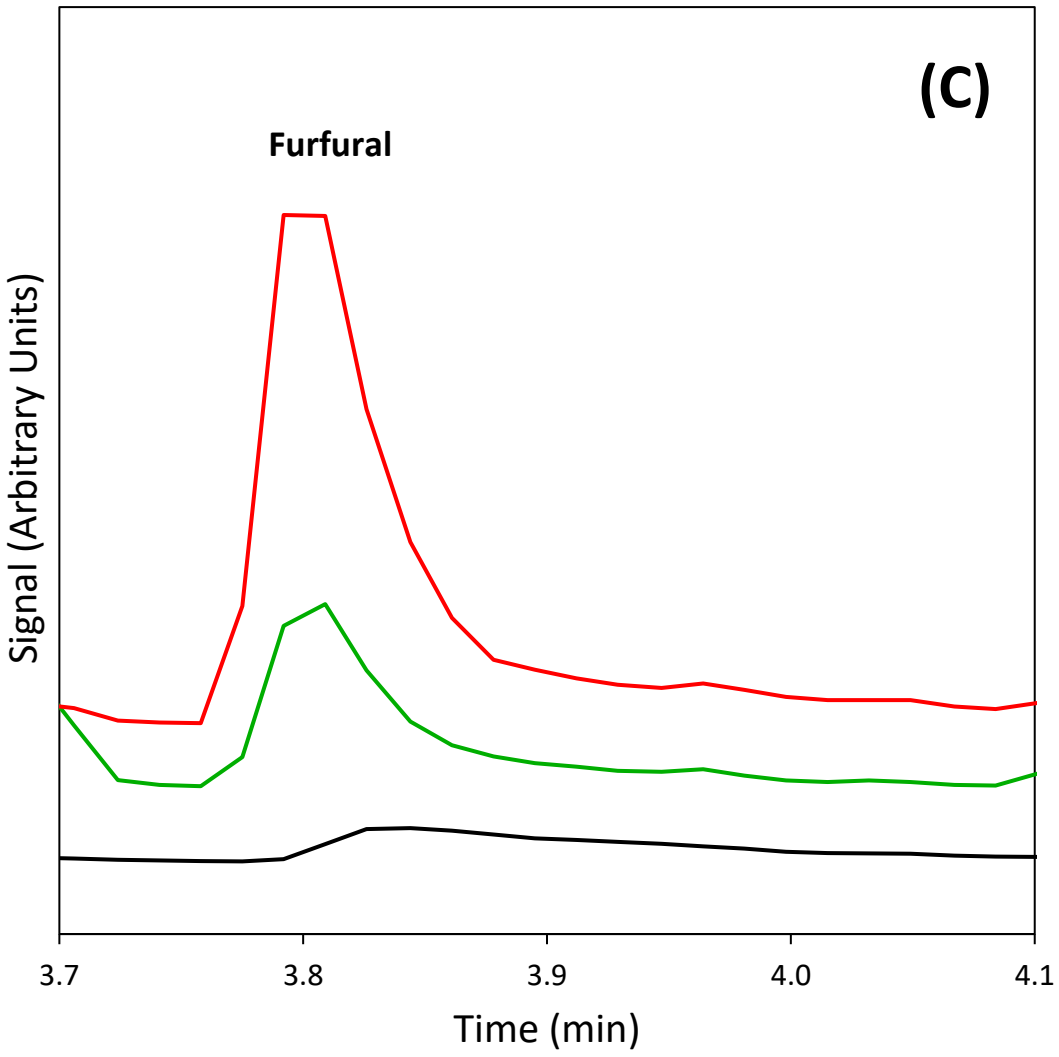
The comparison of ICECLES vs. SBSE was extended using 5, 25 and 50 pg/mL of atrazine. For these concentrations, ICECLES generated signal intensities of >3x, >3x, and >2.5x SBSE, respectively. ICECLES outperformed SBSE for all concentrations of atrazine and performed better at lower atrazine concentrations.

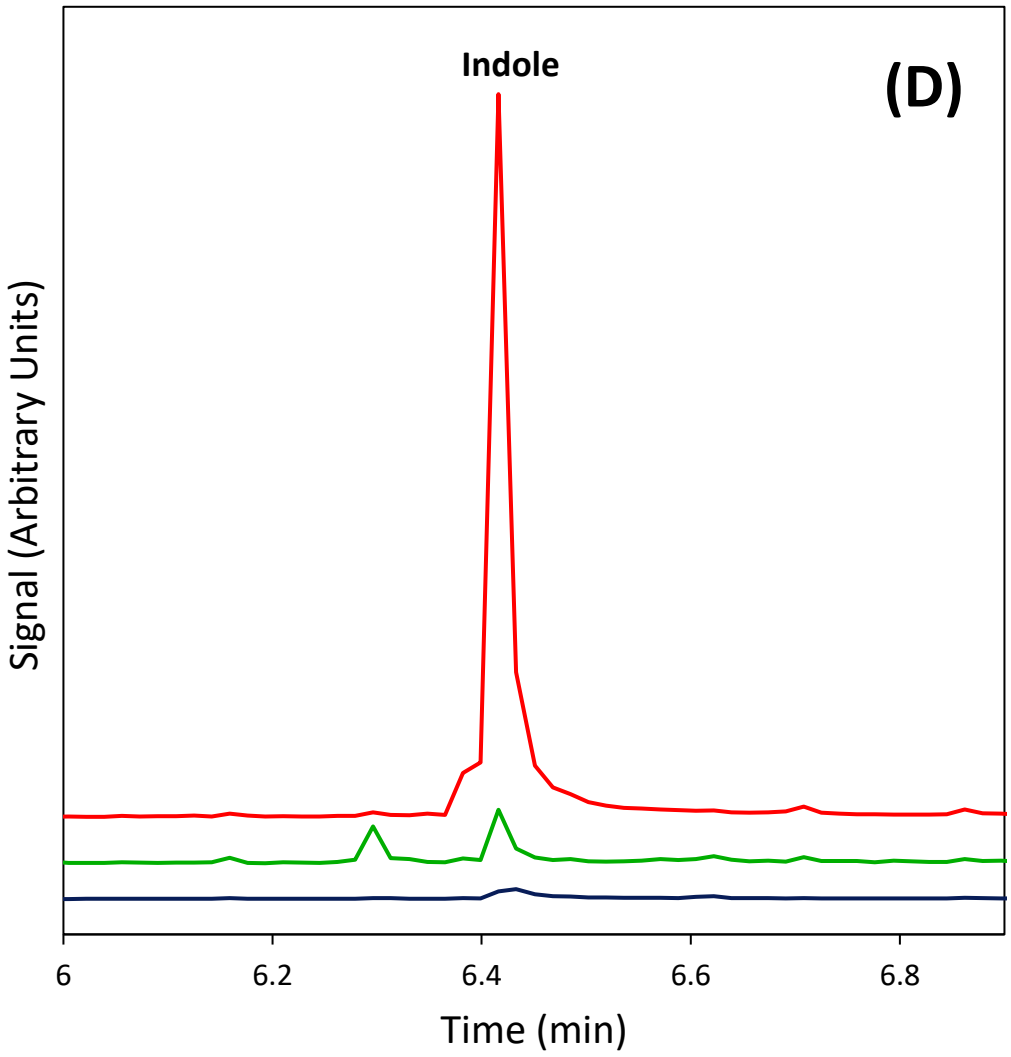
3.3.3 Results for comparison of analytes via TD-GC-MS

Five compounds (furfural, 2-methyl pyrazine, 1-pentanol, indole, and atrazine) with a variety of log K_{ow} values (i.e., 0.41, 0.49, 1.35, 2.05, and 2.70, respectively), were extracted via ICECLES, SBSE, and SPME for TD-GC-MS analysis. The TD-GC-MS chromatograms for these five compounds using each sample preparation technique are shown in Figure 3.4. For each compound analyzed at each concentration tested, ICECLES produced signal intensities greater than those of SBSE and SPME. For example, ICECLES produced ≈2-7x and ≈7-19x higher signals than SBSE and SPME for the compounds tested, respectively.


SPME
SBSE
ICECLES
1-pentanol
(A)
Signal (Arbitrary Units)
3.1
3.2
3.3
3.4
3.5
Time (min)


(B)
2-methylpyrazine
Signal (Arbitrary Units)
3.5
3.6
3.7
3.8
3.9
4
Time (min)


(C)
Furfural
Signal (Arbitrary Units)
3.7
3.8
3.9
4.0
4.1
Time (min)


Indole
(D)
Signal (Arbitrary Units)
6
6.2
6.4
6.6
6.8
Time (min)

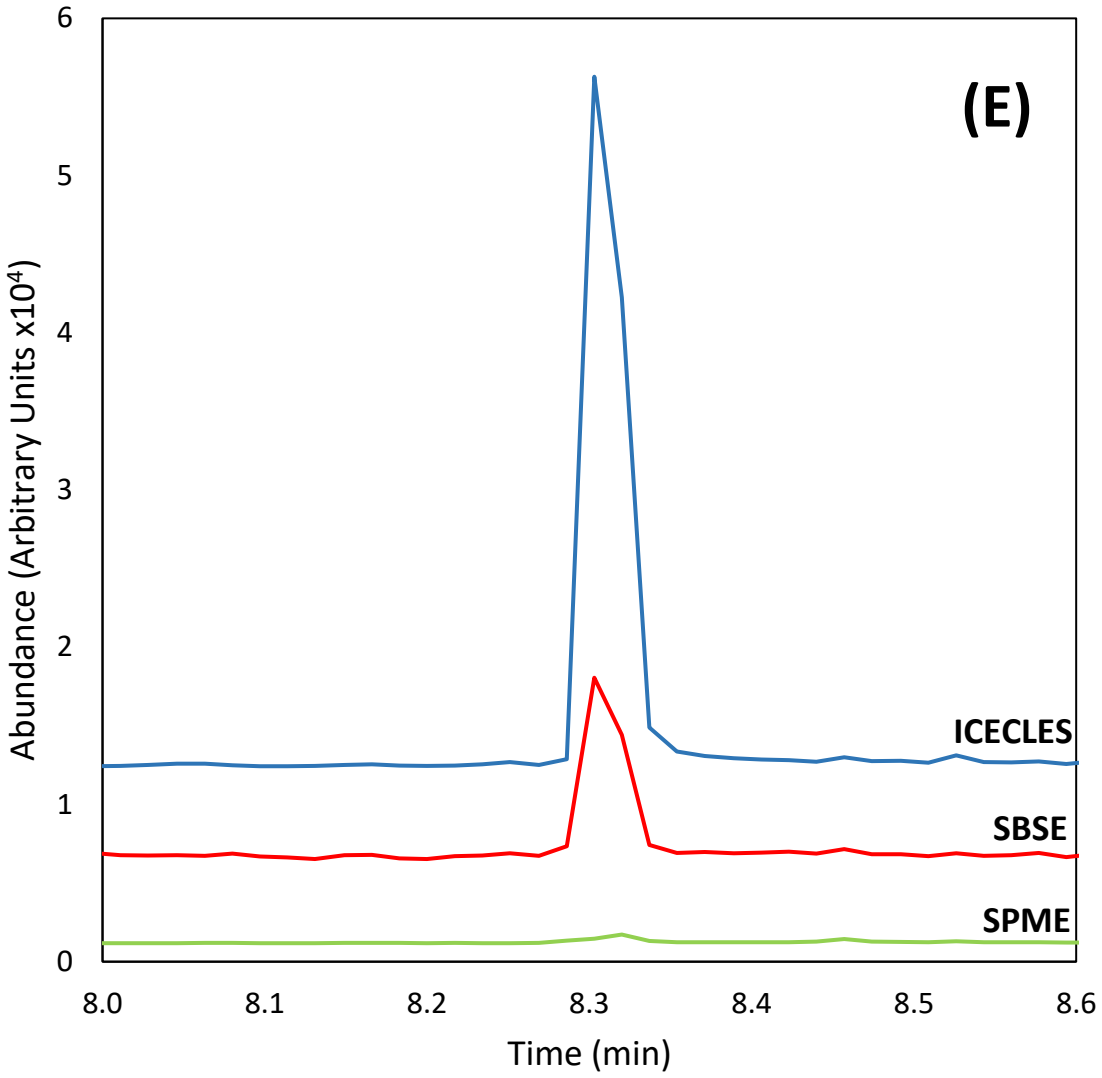


Figure 0.4 Chromatogram comparisons of ICECLES, SBSE, and SPME for aqueous solutions (1 nM) of (A) 1-pentanol (log Kow = 0.41), (B) 2-methyl pyrazine (log Kow = 0.49), (C) furfural (log Kow = 1.35), (D) indole (log Kow = 2.05), and (E) atrazine (log Kow = 2.70). The chromatograms of these preparation techniques are plotted from top to bottom as ICECLES, SBSE, and SPME.

3.3.4 Results for comparison of 60 pesticides in pure and drinking water

Figure 3.5 displays the chromatograms of all 60 pesticides and Table 3.4 presents the concentrations detected ($S/N \geq 3$) for all sixty compounds in purified water. There were a few compounds where SBSE produced greater signal than ICECLES; however, ICECLES was able to detect all 60 pesticides (at 10 ng/mL), with 50 of them detected at or below 0.1 ng/mL. SBSE, SPME (55 µL back-extraction), and SPME (500 µL back-extraction), were able to detect 34, 23, and 8 compounds at or below 0.1 ng/mL, respectively. Moreover, SBSE, SPME (55 µL back-extraction) and SPME (500 µL back-extraction) were unable to detect 5, 8, and 20 compounds, respectively, at any concentration evaluated.

Table 3.5 provides concentrations detected for these sixty compounds in drinking water. ICECLES, SBSE, SPME with 55 µL back-extraction, and SPME with 500 µL back-extraction were able to detect 53, 44, 39, and 31 compounds, respectively at 10 ng/mL, and 32, 25, 13, and 1 compound, respectively, at 0.1 ng/mL. There were eight pesticides that only ICECLES could detect at any concentration tested: aldicarb-sulfoxide, aldicarb-sulfone, amitraz, carbendazim, cycloate, S-Ethyl dipropylthiocarbamate (EPTC), methomyl, and oxamyl.

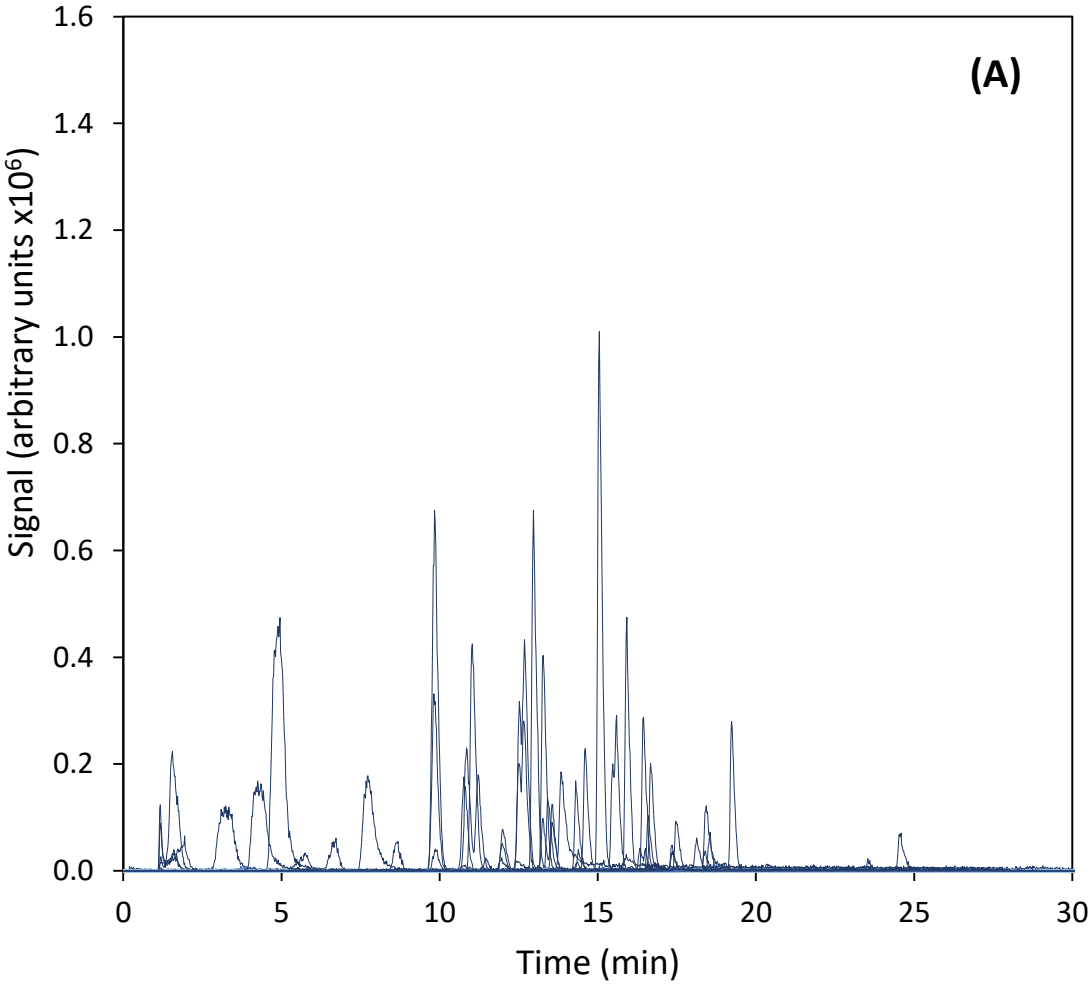
For both purified and drinking water, ICECLES outperformed SBSE and SPME for all pesticides analyzed. The reduced extraction efficiency for each extraction technique going from purified water to drinking water indicates a significant matrix effect for the more complex drinking water sample.

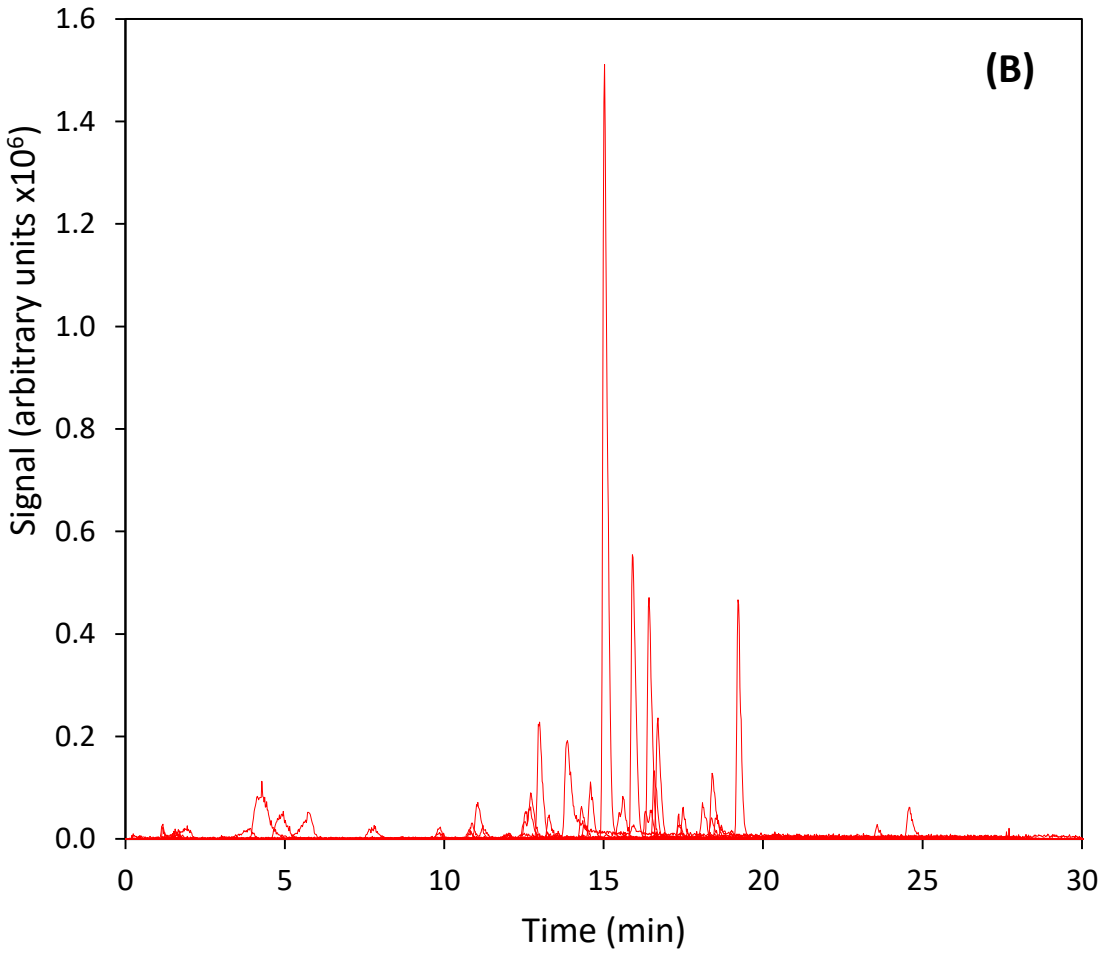
Table 0.5 Comparison of concentrations detected with ICECLES, SBSE, and SPME in deionized water

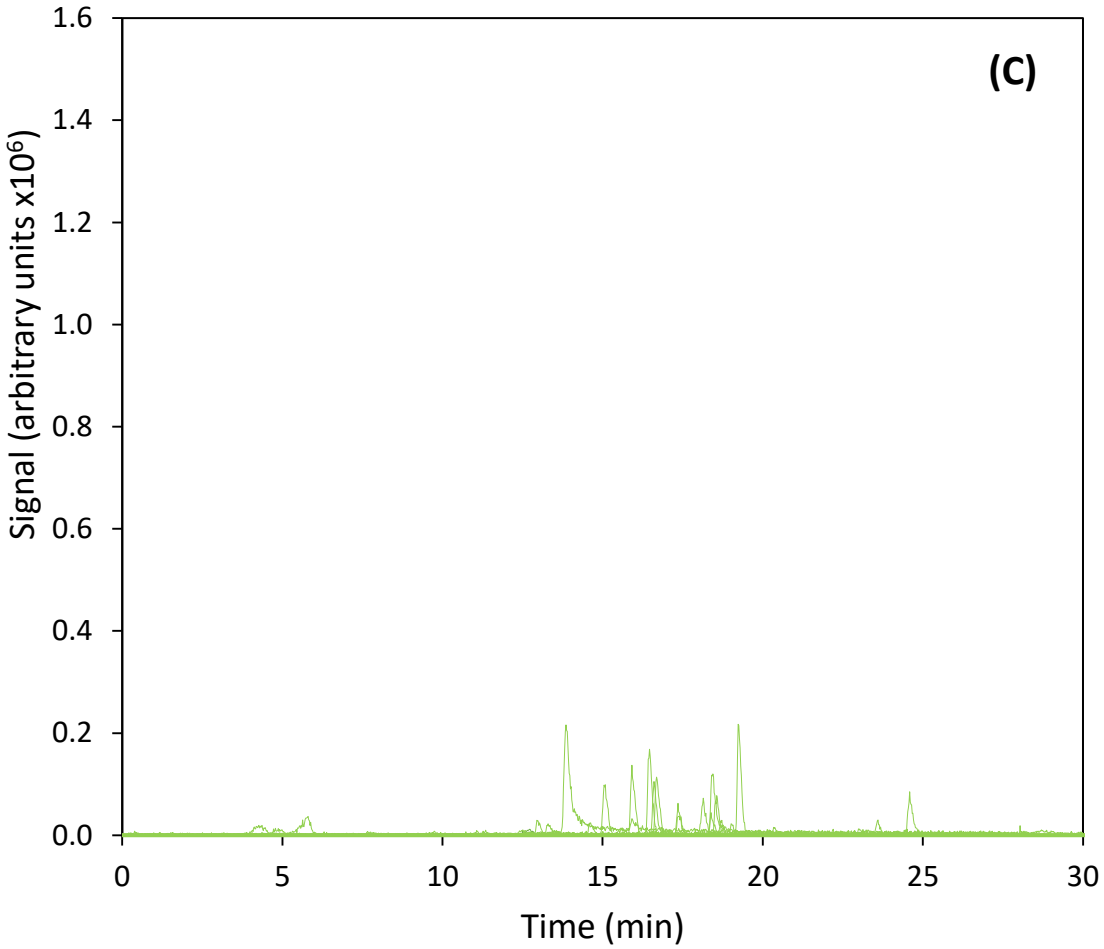
Compound	Log K_{ow}	Concentration Detected (ng/mL)				Compound	Log K_{ow}	Concentration Detected (ng/mL)			
		ICECLES	SBSE	SPME[1]	SPME[2]			ICECLES	SBSE	SPME[1]	SPME[2]
Aldicarb	1.1	10	10	ND[3]	ND	Fuberidazole	2.7	0.1	0.1	1.0	10
Aldicarb-sulfoxide	1.4	1.0	ND	ND	ND	Furathiocarb	4.7	0.1	0.1	0.1	1
Aldicarb-sulfone	1.4	1.0	ND	ND	ND	Iprovalicarb	3.2	0.1	0.1	0.1	1
Aminocarb	1.9	0.1	1.0	1.0	10	Isopropalin	1.4	10	10	10	10
Amitraz	5.5	10	10	ND	ND	Isocarbamid	2.0	0.1	10	10	ND
Acibenzolar-S-Methyl	3.1	0.1	0.1	1.0	10	Mepronil	0.6	0.1	0.1	0.1	1
Bendiocarb	1.7	0.1	1.0	1.0	10	Methiocarb	2.9	10	10	10	10
Butylate	4.2	0.1	0.1	0.1	1	Methomyl	0.1	0.1	ND	ND	ND
Carbaryl	2.4	0.1	0.1	1.0	10	Metolcarb	1.7	0.1	1.0	10	ND
Carbendazim	1.5	0.1	10	10	ND	Napropamide	3.4	0.1	0.1	0.1	0.1
Carbofuran	2.3	0.1	1.0	10	ND	Naproanilide	4.4	0.1	0.1	0.1	0.1
Chlorantraniliprole	2.8	0.1	1.0	10	10	Oxamyl	-0.5	10	ND	ND	ND
Chlorodimeform	2.9	0.1	0.1	1.0	ND	Oxamyl oxime	0.2	10	ND	ND	ND
Chlorbufam	3.6	0.1	0.1	1.0	ND	Oryzalin	3.7	0.1	1.0	10	ND
Chlorpropham	3.8	0.1	0.1	1.0	10	Phenmedipham	2.7	0.1	1.0	1.0	10
Cymiazole	2.5	0.1	0.1	0.1	1	Pirimicarb	1.7	0.1	0.1	1.0	10
Cycloate	3.9	0.1	0.1	0.1	1	Promecarb	3.1	0.1	0.1	0.1	1
Desmedipham	3.2	0.1	0.1	0.1	1	Propamocarb HCl	4.9	0.1	1.0	10	ND
Diallate	3.3	0.1	0.1	0.1	1	Propanil	3.1	0.1	0.1	1.0	10
Dimepiperate	5.6	0.1	0.1	0.1	0.1	Propham	2.6	0.1	1.0	10	ND
Dioxacarb	4.9	0.1	0.1	0.1	1	Thiabendazole	2.5	0.1	0.1	0.1	1
Diphenamid	2.4	0.1	0.1	0.1	1	Thiodicarb	1.6	0.1	1.0	10	ND
EPTC	3.2	0.1	0.1	1.0	10	Triallate	4.6	0.1	0.1	0.1	1
Ethiofencarb	2.0	10	10	10	ND	Trichlamide	5.6	0.1	0.1	0.1	1
Etobenzanid	4.3	0.1	0.1	0.1	1	2,3,5-Trimethacarb	2.5	0.1	1.0	1.0	10
Fenfuram	2.6	0.1	0.1	1.0	10	3,4,5-Trimethacarb	2.6	0.1	1.0	1.0	10
Fenoxycarb	4.3	0.1	0.1	0.1	0.1	Vernolate	3.8	0.1	0.1	0.1	1
Fenthiocarb	3.3	0.1	0.1	0.1	0.1	XMC	2.3	0.1	1.0	1.0	10
Fenoxanil	3.5	0.1	0.1	0.1	1	Xylylcarb	2.1	10	10	ND	ND
Formetanate HCl	4.6	0.1	1.0	10	ND	Zoxamide	3.8	0.1	0.1	0.1	1

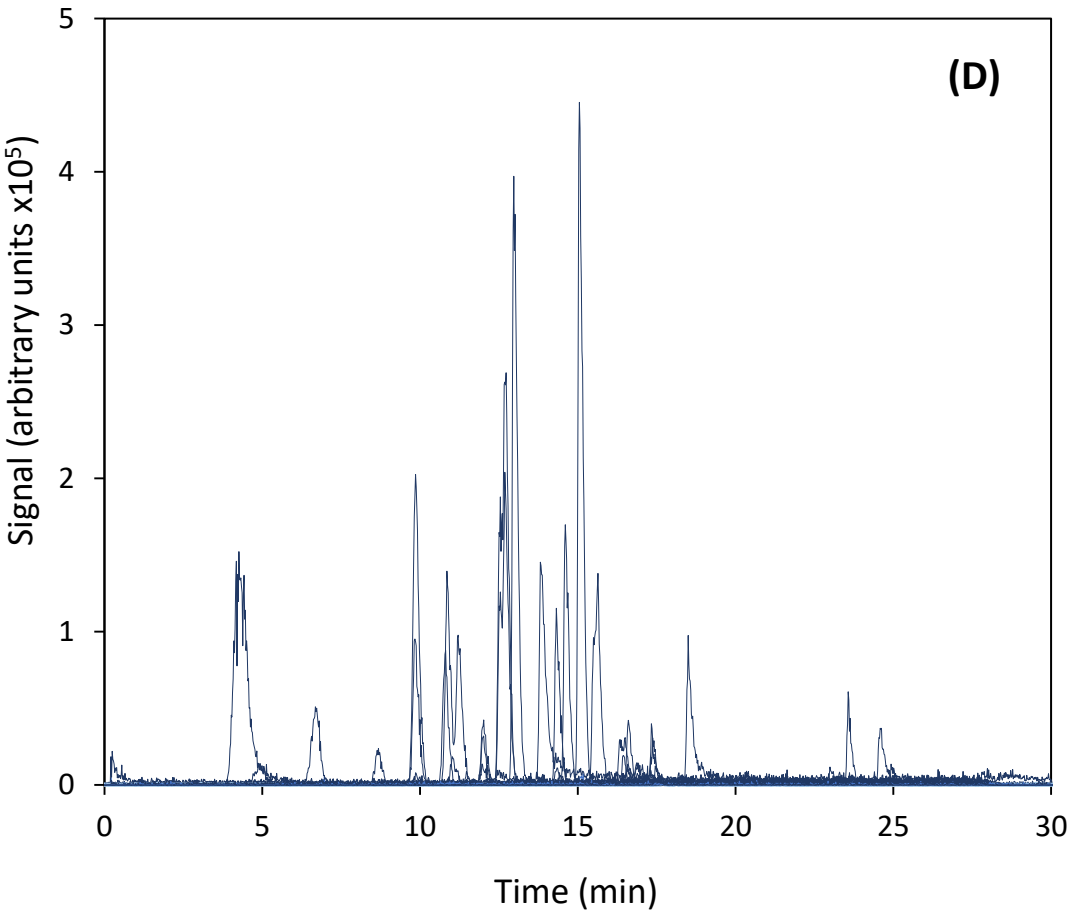
Table 0.6 Comparison of concentrations detected with ICECLES, SBSE, and SPME in drinking/tap water

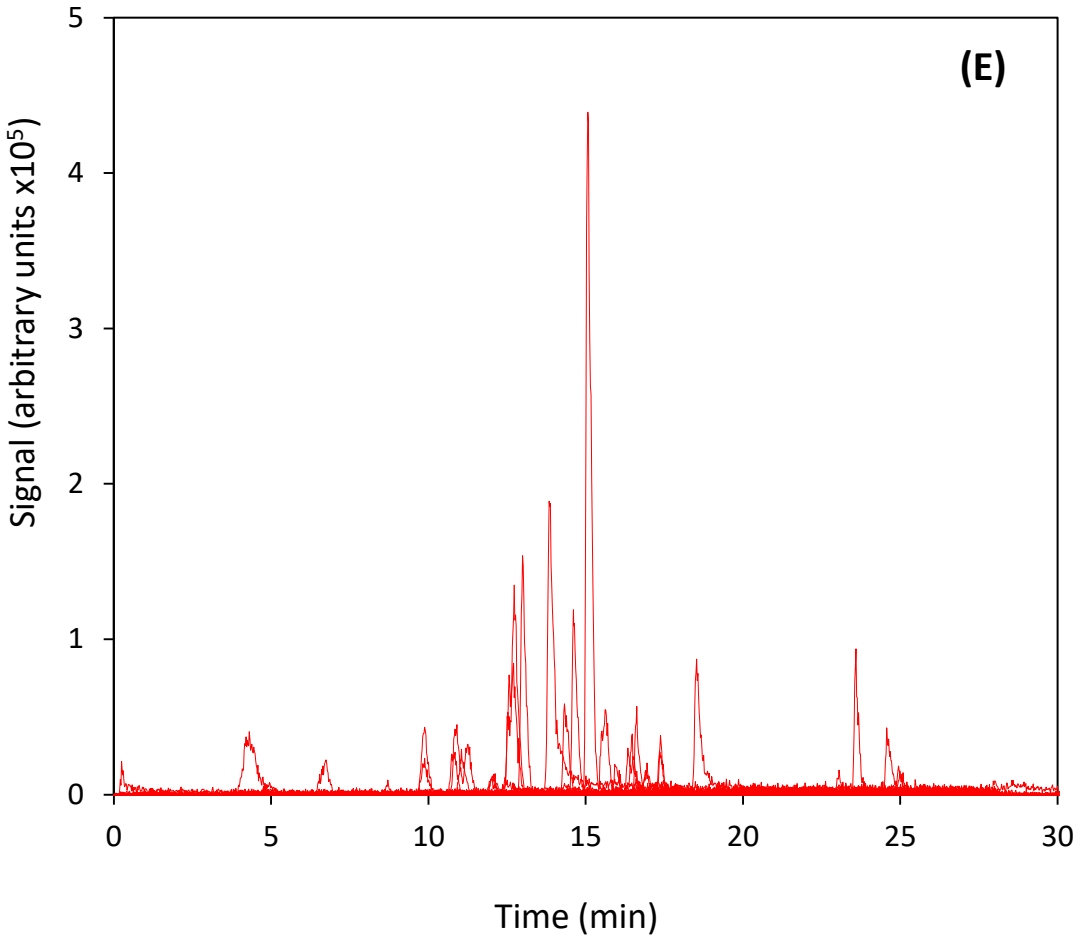
Compound	Log K_{ow}	Concentration Detected (ng/mL)				Compound	Log K_{ow}	Concentration Detected (ng/mL)			
		ICECLES	SBSE	SPME[1]	SPME[2]			ICECLES	SBSE	SPME[1]	SPME[2]
Aldicarb	1.1	ND	ND	ND[3]	ND	Fuberidazole	2.7	0.1	0.1	1	10
Aldicarb-sulfoxide	1.4	10	ND	ND	ND	Furathiocarb	4.7	0.1	1	1	10
Aldicarb-sulfone	1.4	10	ND	ND	ND	Iprovalicarb	3.2	0.1	0.1	0.1	1
Aminocarb	1.9	ND	ND	ND	ND	Isopropalin	1.4	10	10	10	ND
Amitraz	5.5	10	ND	ND	ND	Isocarbamid	2.0	0.1	0.1	1	10
Acibenzolar-S-Methyl	3.1	0.1	0.1	1	10	Mepronil	0.6	0.1	0.1	0.1	0.1
Bendiocarb	1.7	0.1	1	ND	10	Methiocarb	2.9	ND	ND	ND	ND
Butylate	4.2	0.1	1	10	ND	Methomyl	0.1	10	ND	ND	ND
Carbaryl	2.4	0.1	0.1	1	10	Metolcarb	1.7	0.1	1	10	ND
Carbendazim	1.5	10	ND	ND	ND	Napropamide	3.4	0.1	0.1	1	10
Carbofuran	2.3	1	1	10	ND	Naproanilide	4.4	0.1	0.1	0.1	1
Chlorantraniliprole	2.8	0.1	1	1	10	Oxamyl	-0.5	10	ND	ND	ND
Chlorodimeform	2.9	10	10	10	ND	Oxamyl oxime	0.2	ND	ND	ND	ND
Chlorbufam	3.6	0.1	0.1	1	ND	Oryzalin	3.7	1	10	ND	ND
Chlorpropham	3.8	0.1	0.1	1	10	Phenmedipham	2.7	1	10	ND	ND
Cymiazole	2.5	ND	ND	ND	ND	Pirimicarb	1.7	0.1	0.1	1	10
Cycloate	3.9	10	ND	ND	ND	Promecarb	3.1	0.1	0.1	0.1	1
Desmedipham	3.2	1	1	ND	ND	Propamocarb HCl	4.9	ND	ND	ND	ND
Diallate	3.3	1	1	1	10	Propanil	3.1	0.1	0.1	0.1	1
Dimepiperate	5.6	10	10	10	ND	Propham	2.6	0.1	1	10	ND
Dioxacarb	4.9	1	10	ND	ND	Thiabendazole	2.5	0.1	0.1	0.1	1
Diphenamid	2.4	0.1	0.1	0.1	1	Thiodicarb	1.6	0.1	1	1	10
EPTC	3.2	10	ND	ND	ND	Triallate	4.6	1	1	1	10
Ethiofencarb	2.0	ND	ND	ND	ND	Trichlamide	5.6	0.1	0.1	0.1	1
Etobenzanid	4.3	0.1	0.1	0.1	1	2,3,5-Trimethacarb	2.5	0.1	0.1	1	10
Fenfuram	2.6	0.1	0.1	1	10	3,4,5-Trimethacarb	2.6	0.1	0.1	0.1	1
Fenoxycarb	4.3	0.1	0.1	0.1	1	Vernolate	3.8	1	1	1	10
Fenthiocarb	3.3	0.1	0.1	0.1	1	XMC	2.3	0.1	0.1	1	10
Fenoxanil	3.5	0.1	0.1	0.1	1	Xylylcarb	2.1	1	10	ND	ND
Formetanate HCl	4.6	1	1	ND	ND	Zoxamide	3.8	0.1	0.1	1	10


(A)
Signal (arbitrary units x10^6)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
Time (min)


(B)
Signal (arbitrary units x10^6)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
Time (min)


(C)
Signal (arbitrary units x10^6)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
Time (min)


(D)
Signal (arbitrary units x10^5)
0
1
2
3
4
5
0
5
10
15
20
25
30
Time (min)


(E)
Signal (arbitrary units x10^5)
0
1
2
3
4
5
0
5
10
15
20
25
30
Time (min)

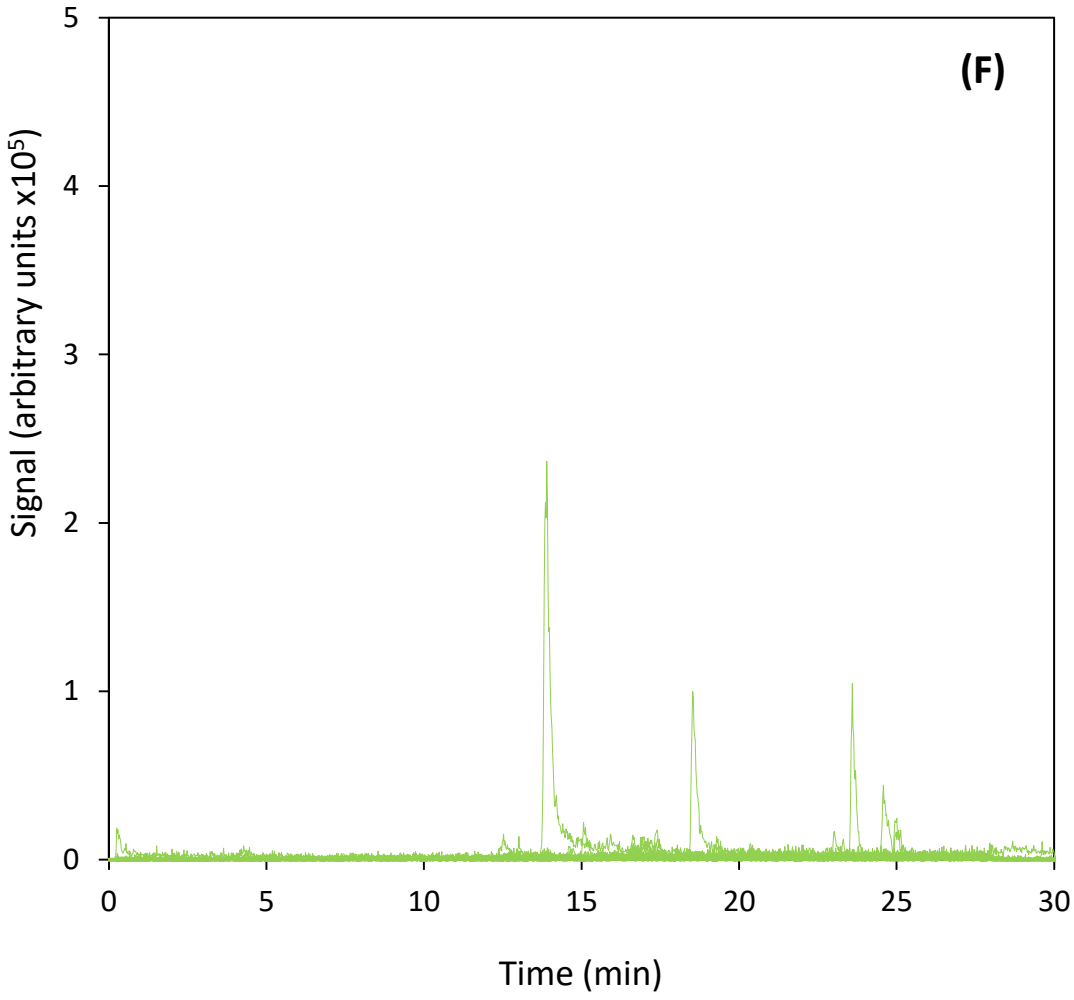
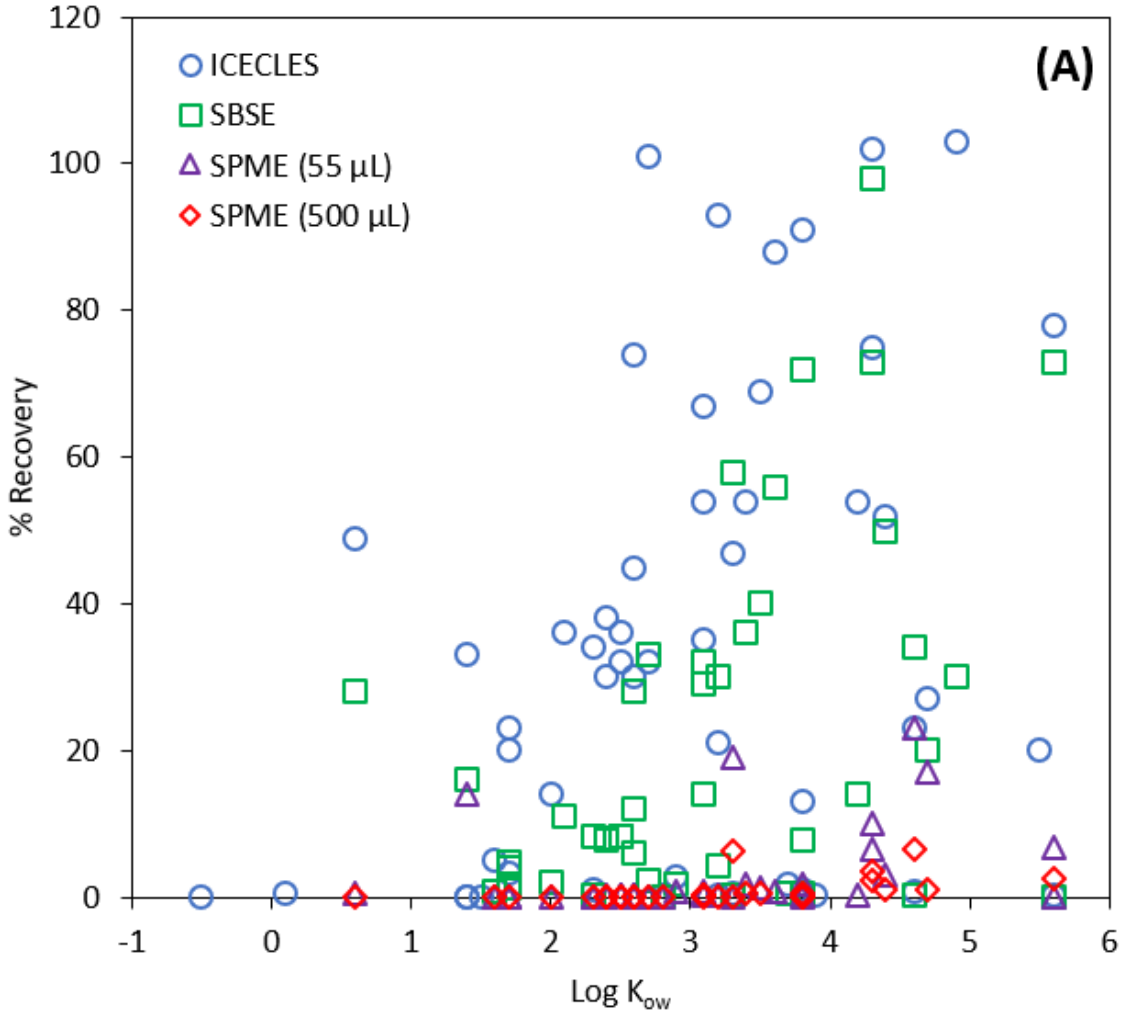
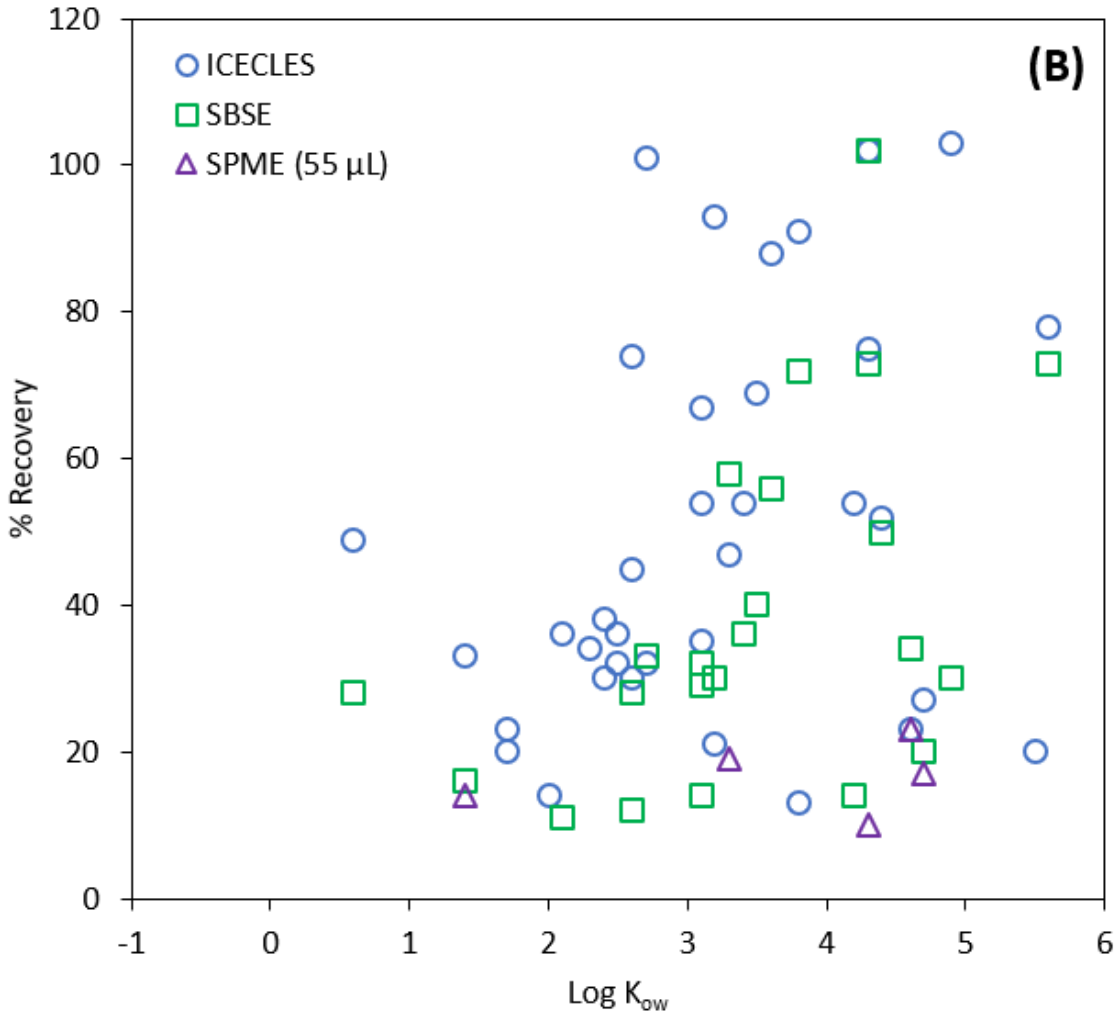


Figure 0.5 LC-MS/MS chromatographic comparison of a suite of pesticides at 0.1 ng/mL. Individual quantification transition chromatograms from extraction of purified water by ICECLES (A), SBSE (B), and SPME (C; back-extraction with 55 µL) and drinking water by ICECLES (D), SBSE (E), and SPME (F; back-extraction with 55 µL) are plotted. Note: Ethiofencarb, which was not detectable at 0.1 ng/mL, was excluded from the figure because the quantification transition produced excessive noise that obscured the other chromatograms. Also note that SPME with 500 µL back-extraction is not shown since it performed worse than back-extraction with 55 µL of solvent for all analytes.

It should be noted that previous studies [73,74,75,76] have generated lower detection of pesticides in drinking water than the work presented here; however, this work is meant to be a comparison of extraction efficiencies of multiple techniques. It could be feasible, for future work, to attempt lower detections of these pesticides with ICECLES.

ICECLES allowed LC-MS/MS detection of all 60 pesticides evaluated at 10 ng/mL in purified water, with 50 pesticides detected at or below 0.1 ng/mL, compared to 34 and 23 for SBSE and SPME, respectively. Furthermore, in drinking water, 32, 25, and 13 pesticides were detected via LC-MS/MS at 0.1 ng/mL by ICECLES, SBSE, and SPME, respectively. Overall, ICECLES consistently produced better extraction efficiencies than the other extraction techniques evaluated. Figure 3.6 depicts the recoveries of each pesticide as a function of the log Kow for ICECLES, SBSE, SPME (with 500 µL back extract), and SPME (with 55 µL back extract).


(A)
ICECLES
SBSE
SPME (55 µL)
SPME (500 µL)
% Recovery
Log K_{ow}
120
100
80
60
40
20
0
-1
0
1
2
3
4
5
6


(B)
ICECLES
SBSE
SPME (55 µL)
% Recovery
Log K_{ow}
120
100
80
60
40
20
0
-1
0
1
2
3
4
5
6

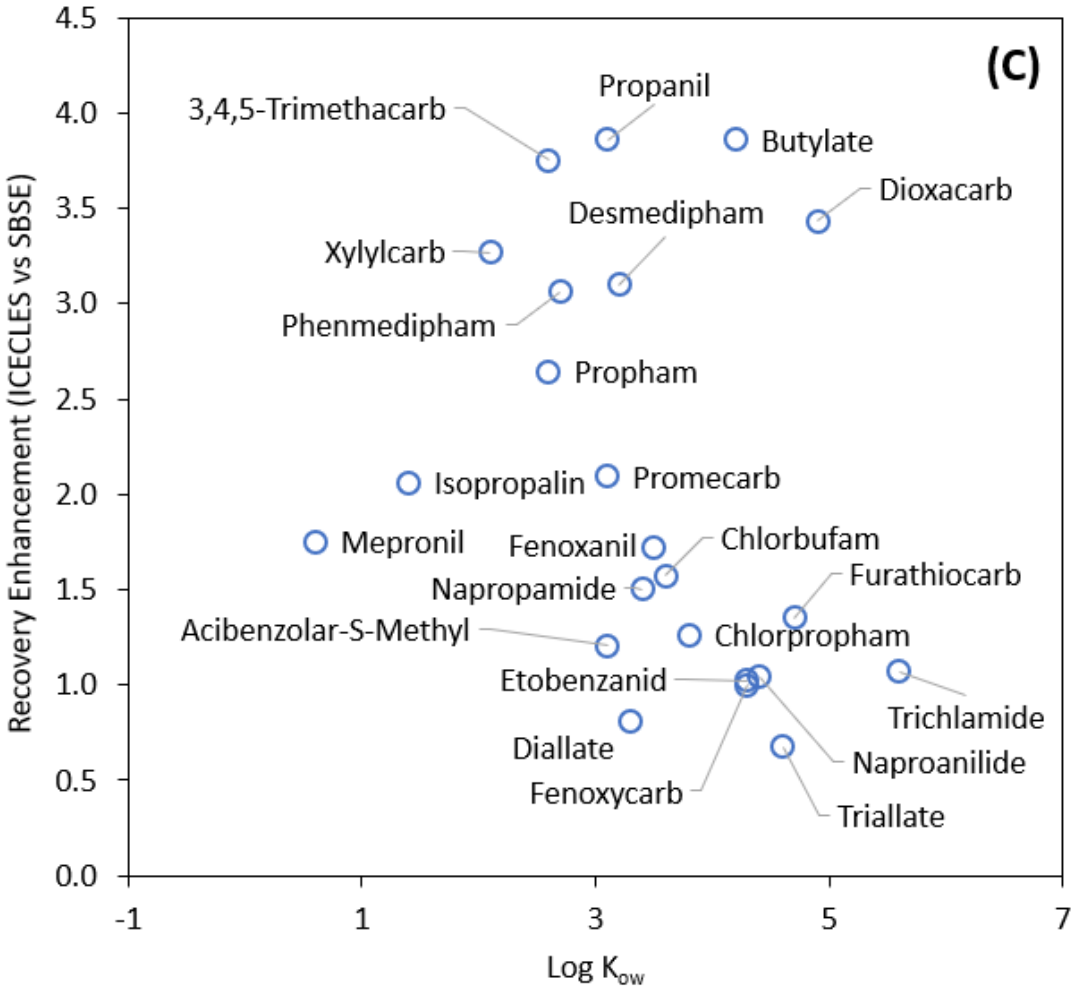
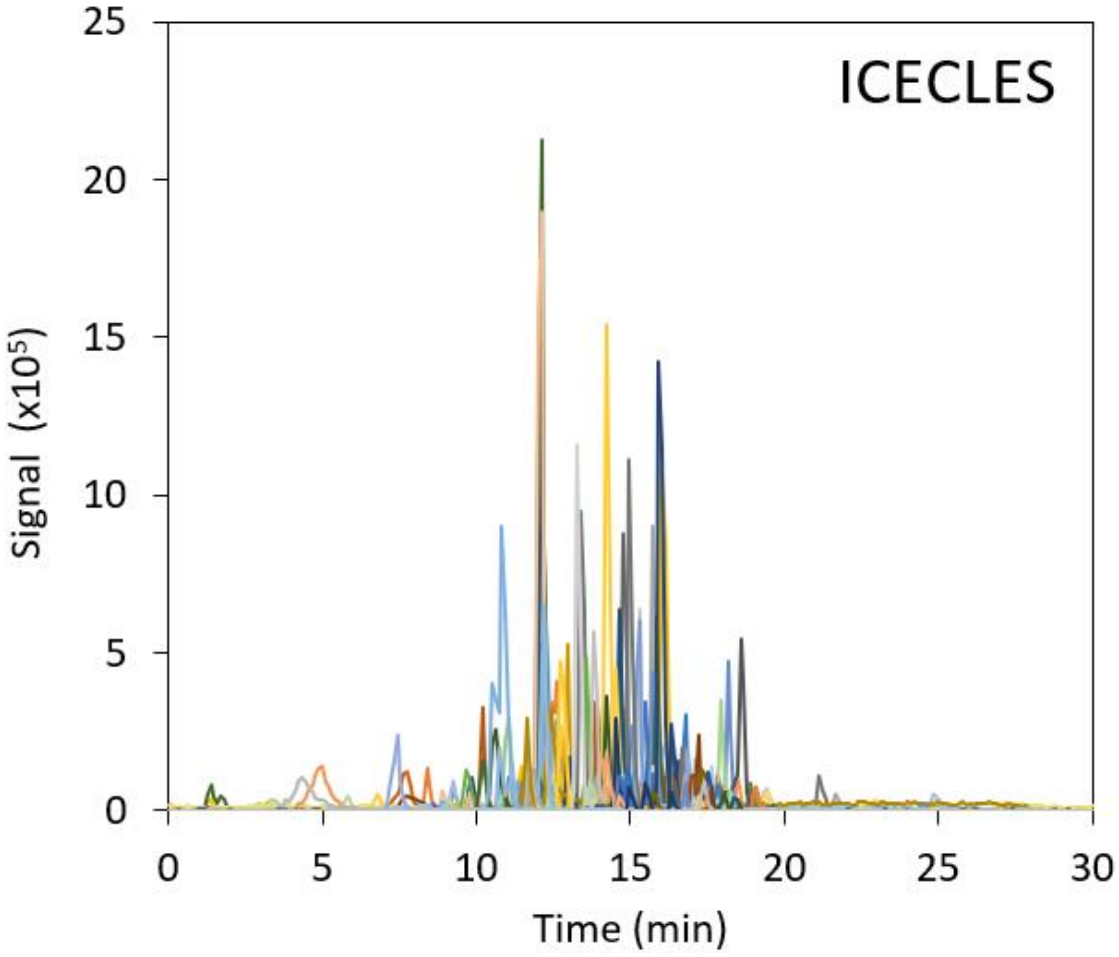
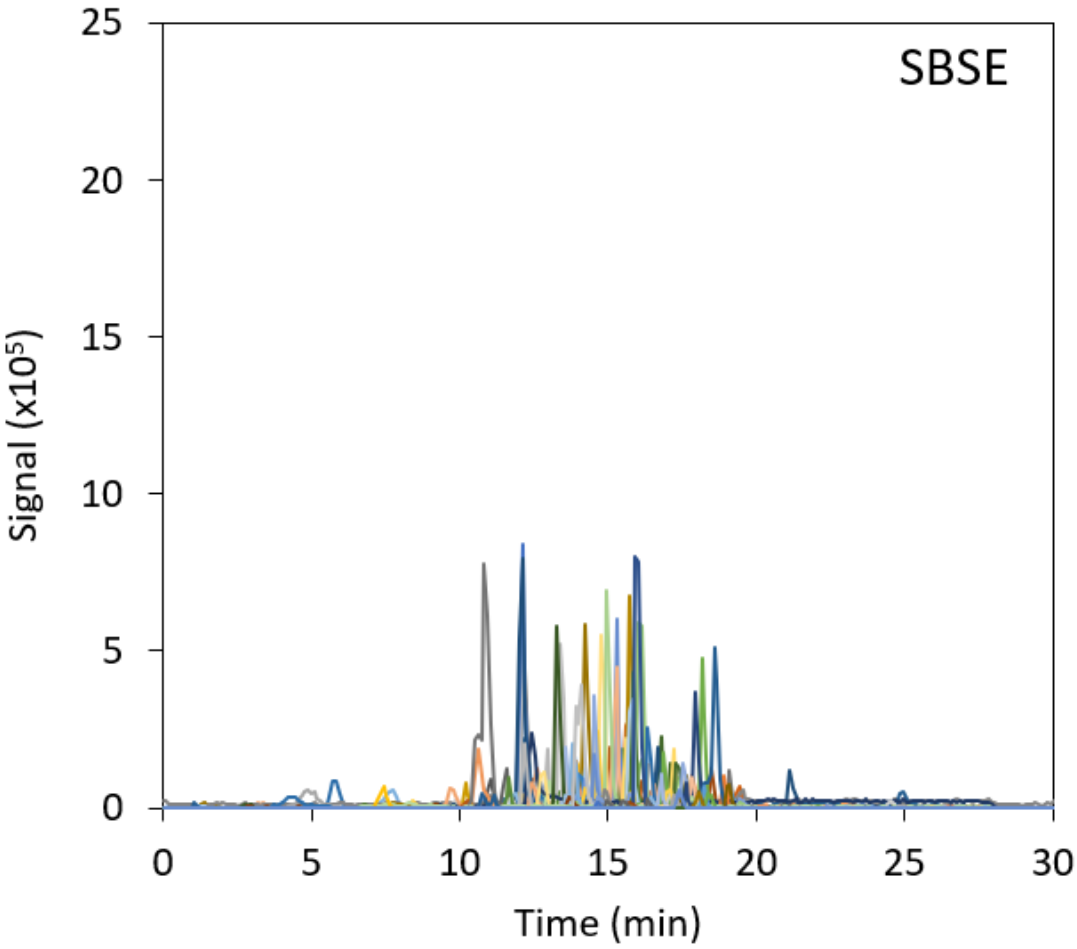


Figure 0.6 The recoveries of each pesticide as a function of the log K_{ow} for ICECLES, SBSE, SPME (with 500 µL back extract), and SPME (with 55 µL back extract). A) The recovery of each pesticide for each extraction technique is plotted. B) Only recoveries of 10% or above are plotted to clarify the results of extraction with of each pesticide. C) The recovery enhancement of ICECLES over SBSE (i.e., the recovery found with ICECLES divided by the recovery produced using SBSE) for those compounds that produced recoveries above 10% for both ICECLES and SBSE. Recovery enhancements of ICECLES over SPME were not plotted since there are too few compounds (N = 5) for which SPME produced recoveries greater than 10% to evaluate a meaningful trend.

3.3.5 Results for comparison of 313 pesticides in pure water

ICECLES was able to detect (S/N ≥ 3) 273 of the 313 compounds at 0.1 ng/mL, versus 229 and 192 for SBSE and SPME, respectively. Figure 3.7 shows the chromatograms of ICECLES, SBSE, and SPME, respectively, and demonstrates visually ICECLES' ability to outperform other microextraction techniques[56].




SBSE
Signal (x10⁵)
25
20
15
10
5
0
0
5
10
15
20
25
30
Time (min)

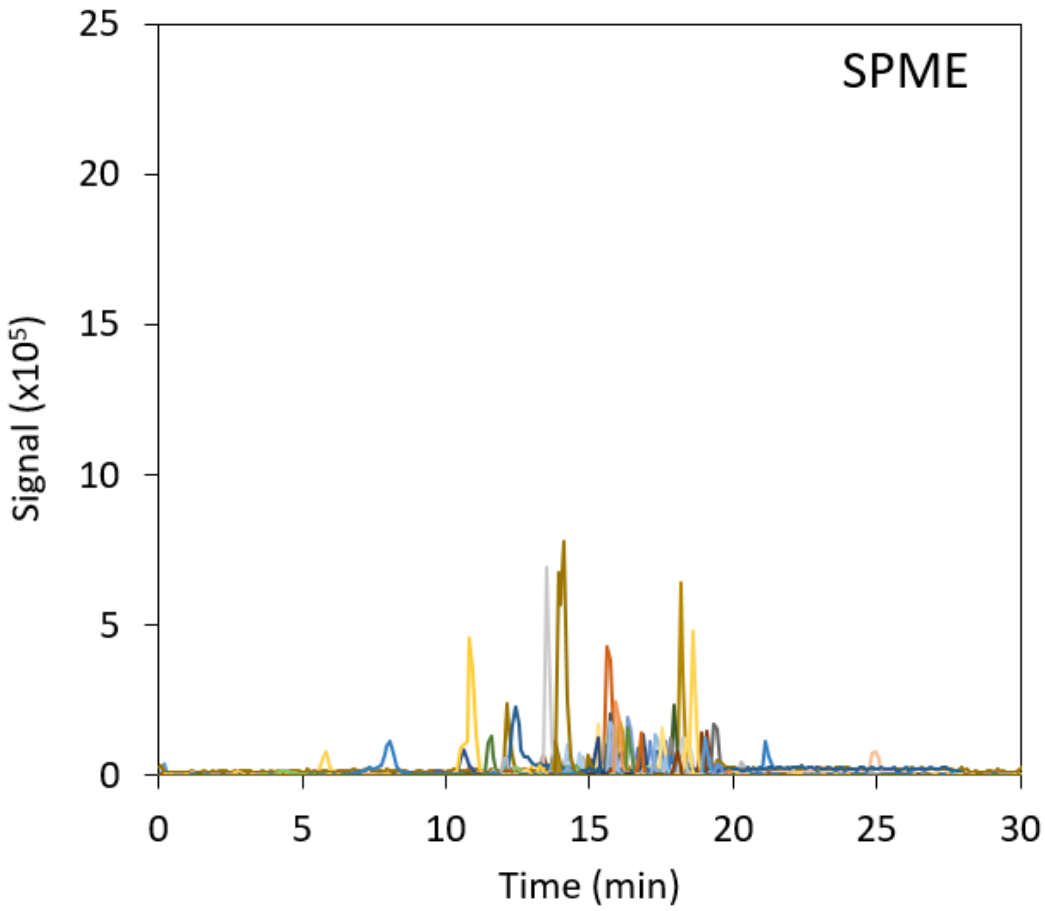


Figure 0.7 Chromatograms of a suite of pesticides (N = 313, each spiked at 100 pg/mL) extracted from a water sample (10-mL) using ICECLES, SBSE, and SPME. ICECLES extraction produced increased signals for the majority of the pesticides analyzed.

3.3.6 Conclusion

ICECLES demonstrated excellent extraction efficiencies for multiple compounds over a wide range of log K_{ow} values from water. ICECLES outperformed all other sample preparation techniques regardless of the instrumentation used (TD-GC-MS and/or LC-MS/MS). ICECLES is greener (i.e., produces less organic solvent waste) than some sample preparation techniques (i.e., LLE and SPE), provides better limits of detection, and minimizes sample volumes. The versatility of ICECLES allows for desorption via TD-GC or back-extraction into LC-MS/MS-amenable solvents, a rare trait for microextraction techniques. ICECLES offers a sample preparation alternative that is automated, requires minimal solvent, and excels at simultaneously extracting both non-polar and intermediate polarity analytes.

Chapter 4. Ultratrace analysis of per- and polyfluoroalkyl substances in drinking water using Ice Concentration Linked with Extractive Stirrer (ICECLES) and HPLC-MS/MS

4.1 Introduction

Detection of drinking water contaminants is vital to the protection of human health. One group of contaminants that have recently generated serious concerns over health risks are per- and polyfluoroalkyl substances (PFAS). These compounds are very bio-persistent, leading to their detection in all types of water sources, including drinking water. While analysis of drinking water for PFAS is important, it is currently arduous to detect ultratrace levels of these contaminants. Specifically, current ultratrace PFAS analysis methods are difficult, costly, require large sample volumes, and consume relatively large volumes of organic solvent. In the present work, an analytical method using Ice Concentration Linked with Extractive Stirrer (ICECLES) and high performance liquid chromatography—tandem quadrupole mass spectrometry (HPLC-MS/MS), was developed and validated to provide simple and ultratrace analysis of drinking water for 14 PFAS. The method featured a relatively low sample volume requirement (10 mL), automated extraction, minimal matrix effects, and green sample processing (i.e., the method requires only 50 µL of methanol per sample). The method produced a wide linear range of 0.5 to 500 ng/L, ultratrace limits of detection (0.05 to 0.3 ng/L), and good accuracy and precision (i.e., 87 to 108% accuracy and ≤19% relative standard deviation as a measure of precision). This method was tested on drinking water samples from across the United States and detected at least one PFAS compound in 12 of the 14 drinking water samples tested. Perfluorooctanoic acid (PFOA), perfluorohexanoic acid

(PFHxA), and perfluorobutanesulfonic acid (PFBS) were detected in 86, 80, and 71% of the samples tested with maximum concentrations of 213 ng/L for PFOA, 40.4 ng/L for PFHxA, and 2.22 ng/L for PFBS. Additionally, perfluorononanoic acid, perfluorodecanoic acid, and perfluoroheptanoic acid were each detected in at least one drinking water sample at concentrations > 20 ng/L. The availability of the method presented here allows ultratrace detection of PFAS while circumventing many of the disadvantages of current methods.

Since their creation in the 1940s, per- and polyfluoroalkyl substances (PFAS) have been utilized for various industrial products, such as fluoropolymers, fluororepellants, and fluorosurfactants.[20] PFAS are used in medical devices, electronics (e.g., mobile phones), non-stick cookware, carpet, Class B firefighting foam, and furniture upholstery. While PFAS are very useful materials, studies have shown that they are extremely persistent, readily bioaccumulate, are present in various water sources [21-30] and exposure to PFAS leads to adverse health effects.

PFAS have been shown to produce many adverse health effects. In fact, studies performed by the U.S. Department of Health and Human Services showed PFAS lead to high cholesterol, low birth weight, immune system dysfunction, thyroid hormone disruption, tumor development, and cancer.[77] These adverse effects are of great concern, as the general population can be exposed to PFAS through indoor environments, food, and water. In the early 2000s, the EPA and several industry partners who produce PFAS compounds agreed that the discontinuation of some “long-changed” PFAS compounds was necessary.[78, 79] Additionally, the EPA has developed a lifetime health advisory level for two of the more prevalent and problematic PFAS, perfluorooctanoic acid (PFOA) and

perfluorooctane sulfonic acid (PFOS), of 70 ng/L in drinking water and has issued a Groundwater Guidance and Maximum Contaminant Level (MCL) for interagency review.[31]

Because of the large amount of drinking water humans consume, along with PFAS toxicity and their ability to bioaccumulate, ultratrace detection of these compounds is increasingly important, but currently very difficult. Multiple review publications [21, 80] have aggregated the current analytical methods for PFAS in drinking water. Selected representative methods are listed in Table 4.3 and a comparison of method LODs are listed in Table 4.4. While most methods listed in Table 4.3 were able to detect PFAS at ultratrace concentrations, they each have major disadvantages, including arduous sample preparation and large organic solvent volume requirements. The overwhelming majority of these methods use solid-phase extraction (SPE)[81-89] because of its ability to increase concentration factors by slowly passing very large volumes of drinking water (100 to 5000 mL), through an SPE column, eluting PFAS with organic solvent, evaporating the solvent, and then reconstituting the residue in a small volume of aqueous solvent for analysis. Depending on the analyte, concentration factors of 1,000 could theoretically be achieved for a 1000 mL sample reconstituted in 1 mL of solvent. While excellent concentration factors can be achieved via SPE, the addition of large volumes of sample through a small column (i.e., 1 to 3 mL typical column capacity) is a time-consuming process which requires constant supervision if done manually. While an automated SPE method[90] is available, it generated higher LODs than its offline counterparts and required specialized equipment. While there are SBSE[91] and SPME[92] methods requiring only 10-

20 mL of sample, they require custom synthesis of sorbents, solvent evaporation, and the SBSE method requires a relatively large volume of organic solvent.

First described by Maslamani et al.,[45] ICECLES combines two techniques: SBSE and freeze concentration (FC). ICECLES provides a simple and automated microextraction alternative that minimizes matrix interference and preconcentrates analytes. ICECLES has performed well for the extraction of pesticides from drinking water, comparing favorably to SBSE and SPME.[56] ICECLES was also utilized to detect ultratrace concentrations of nitrosodipropylamine (i.e., a difficult to analyze carcinogen) at an LOD of 0.2 ng/L in drinking water.[4] While ICECLES is well-suited for drinking water analysis, it has also been used for the analysis of more complex matrices to determine food/flavor compounds from green tea,[3] and atrazine at ultratrace levels (i.e., LOD = 8 ng/kg) from soil.[93]

The goal of this study was to evaluate the ability of ICECLES to extract PFAS from drinking water to allow ultratrace detection via HPLC-MS/MS, while providing a simple, automated, and green method to address the disadvantages of current, mainly SPE-based, techniques. Since ICECLES has proven well-suited to detect analytes at ultratrace concentrations and has shown an ability to minimize matrix effects, it was expected that ICECLES would address the disadvantages of current ultratrace PFAS analysis methods, while producing ultratrace PFAS detection. A secondary goal of the study was to determine the prevalence of PFAS in drinking water sources across the U.S.

4.2 Experimental

The following sections describe the materials and methods used for the analysis of PFAS from drinking water using ICECLES-HPLC-MS/MS.

4.2.1 Materials and standards

Methanol (LC-MS grade), acetonitrile (LC-MS grade), and ammonium acetate (LC-MS grade) were purchased from Fisher Scientific (Waltham, MA). The stir bars used for ICECLES were Gerstel Twister^{TM} stir bars (Lot #: 020120161117) with 10 mm length and 0.5-mm film thickness. The individual PFAS selected for the current method were based on the EPA's Method 537.1.[94] A mixed stock standard (1 mL) of these 14 PFAS in acetonitrile:water (95:5, v:v) was purchased from AccuStandard (New Haven, CT) containing N-ethyl perfluorooctane sulfonamideo acetic acid (N-EtFOSAA), N-methyl perfluorooctane sulfonamideo acetic acid (N-MeFOSAA), perfluorobutanesulfonic acid (PFBS), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonic acid (PFHxS), perfluorohexanoic acid (PFHxA), perfluorononanoic acid (PFNA), perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorotetradecanoic acid (PFTeDA), perfluorotridecanoic acid (PFTrDA), and perfluoroundecanoic acid (PFUdA) each at 50 mg/L. From this mix, intermediate standard solutions were prepared in purified water at 10 mg/L and 100 µg/L, by serial dilutions. From these intermediate standard solutions, working solutions (0.5 to 500 ng/L) were prepared in purified water.

The internal standards were also received as a mixture, in 1 mL of methanol:water (99:1, v:v), purchased from Wellington Laboratories (Guelph, Ontario). The mixture

contained 12 mass-labelled PFAS each at 2 mg/L: perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid (PFBA-$^{13}C_4$), perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid (PFHxA-$^{13}C_5$), perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid (PFHpA-$^{13}C_4$), perfluoro-n-[$^{13}C_8$]octanoic acid (PFOA-$^{13}C_8$), perfluoro-n-[$^{13}C_9$]nonanoic acid (PFNA-$^{13}C_9$), perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid (PFDA-$^{13}C_6$), perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid (PFUdA-$^{13}C_7$), perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid (PFDoA-$^{13}C_2$), perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid (PFTeDA-$^{13}C_2$), sodium perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate (PFBS-$^{13}C_3$), sodium perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate (PFHxS-$^{13}C_3$), and sodium perfluoro-[$^{13}C_8$]octanesulfonate (PFOS-$^{13}C_8$). The perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid internal standard wasn't used for quantitation. The PFTeDA-$^{13}C_2$ internal standard was used for generating signal ratios for PFTrDA. Both N-EtFOSAA and N-MeFOSAA did not require internal standard for quantitation.

In order to create fortified drinking water standards, drinking water (10 mL, pre-screened for PFAS) was transferred into a 15-mL centrifuge tube and fortified at a desired concentration by adding 100 µL of a working standard. From this mixture, the working internal standard solution was created via dilution with purified water to 1.0 µg/L. The working IS solution was added to each sample, standard, and blank, resulting in a final concentration of 10 ng/L. The sample was then mixed briefly and left to stand for approximately 15 min prior to analysis.

4.2.2 Drinking water samples and standards

Because PFAS are present in many drinking water sources,[21-30] the samples were pre-screened for all the above-mentioned PFAS prior to using them for method development and validation. Samples that produced undetectable levels of PFAS were

used for this purpose. Specifically, none of the PFAS analyzed were detected in the Brookings, South Dakota residential tap water; therefore, this tap water source was used for method development, validation, quality controls, and calibration. In order to evaluate the potential for PFAS leaching into the method development drinking water from the storage containers over time, a sample of this water was stored in a 1-L HDPE bottle for more than four months and then tested for PFAS.

Water samples for analysis via the validated method were collected via the sampling protocol described above in multiple locations across the U.S. (Table 4.8 and Figure 4.1). Drinking water samples for method development or analysis were collected from a tap by allowing cold drinking water to run freely at maximum flow for at least 3 min, rinsing a 1-L HDPE bottle with tap water, discarding the rinse solution, collecting cold tap water in the HDPE bottle, and tightly capping the bottle. The drinking water samples were shipped to SGS (Brookings, SD) and stored in a refrigerator until analysis.

4.2.3 ICECLES

A blank, fortified, or field drinking water sample (10 mL) was added to a 20-mL scintillation vial with a sorptive stir bar, the vial was capped and placed in the ICECLES apparatus (described by Maslamani, et al).[45] The ICECLES apparatus was cooled at -5 °C until the sample was completely frozen. The stir speed was initially set to 1200 rpm but was reduced to 300 rpm after approximately 2 hr to reduce “splashing” of the sample solution onto the walls of the vial. Once the sample had completely frozen, the stir bar was removed and placed into an HPLC vial insert. Several parameters were optimized for the stir bar back-extract post-ICECLES, including (1) the desorption solvent, (2) the volume of desorption solvent, and (3) the desorption time. The back-extraction solvents

were chosen based on solubility of the analytes of interest and amenability to HPLC-MS/MS. Several iterations of back-extraction were completed simultaneously to optimize the back-extraction conditions. Back-extraction with the smaller volumes tested (100 and 200 µL) was performed in an HPLC vial insert. The larger solvent volumes (300 and 500 µL) were tested in a 2-mL centrifuge tube.

4.2.4 Liquid chromatography—tandem mass spectrometry

Analysis of PFAS from drinking water was carried out by a Shimadzu Nexera XR HPLC (Tokyo, Japan), and a Sciex 6500+ MS/MS (Redwood City, CA). A delay column (Eclipse XDB-C18, 4.6 x 100 mm, 5 µm) was used to capture (delay) PFAS originating from the HPLC system. This column was plumbed between the mobile phase mixer and the autosampler. An Agilent Poroshell C18 column (4.6 x 50 mm, 2.7 µm), 5 mM ammonium acetate in water as Mobile Phase A, and 100% methanol as Mobile Phase B were used to separate the PFAS. The mobile phase composition was initially held constant at 30% B for 2.0 min, then linearly increased to 90% B over 3.0 min, held constant for 5.0 min, decreased back to 30% B over 0.1 min, and then held constant for 3.9 min for a 14-min total run time, with a flow rate of 0.6 mL/min. The mobile phase gradient was not only optimized for analyte peak shape and selectivity, but also for elution times of delayed PFAS, such that the PFAS originating from the HPLC system did not interfere with PFAS originating from the drinking water sample. The column and autosampler temperatures were 50 °C and 10 °C, respectively. An injection volume of 75 µL was used with 500 µL of an autosampler rinsing solution (acetonitrile:IPA:methanol (1:1:1)) used before and after aspiration. The MS/MS was operated at 350 °C, with an Ion Spray voltage of -4500 V, ion source gas 1 of 50 psi, ion source gas 2 of 50 psi, curtain

gas at 20 psi, and collision gas at 9 psi. The various mass spectrometry parameters for PFAS and their internal standards can be found in Tables 4.1 and 4.2, respectively.

Table 3.1 Mass spectrometry parameters for PFAS

Analyte[b]	*m/z*	Declustering Potential (volts)	Entrance Potential (volts)	Collision Energy (volts)	Cell Exit Potential (volts)
N-EtFOSAA	584 → 419	-60	-10	-28	-21
N-EtFOSAA[1]	584 → 526	-60	-10	-28	-17
N-MeFOSAA	570 → 419	-65	-10	-28	-23
N-MeFOSAA[a]	570 → 483	-65	-10	-22	-15
PFBS	299 → 80	-45	-10	-62	-9
PFBS[a]	299 → 99	-45	-10	-36	-11
PFDA	513 → 469	-35	-10	-16	-15
PFDA[a]	513 → 219	-35	-10	-26	-11
PFDoA	613 → 569	-40	-10	-18	-19
PFDoA[a]	613 → 169	-40	-10	-34	-9
PFHpA	363 → 319	-20	-10	-14	-17
PFHpA[a]	363 → 169	-20	-10	-24	-19
PFHxS	399 → 80	-80	-10	-84	-9
PFHxS[a]	399 → 99	-80	-10	-72	-11
PFHxA	313 → 269	-20	-10	-12	-15
PFHxA[a]	313 → 119	-20	-10	-26	-13
PFNA	463 → 419	-30	-10	-16	-13
PFNA[a]	463 → 219	-30	-10	-24	-11
PFOS	499 → 99	-80	-10	-92	-11
PFOS[a]	499 → 80	-80	-10	-110	-9
PFOA	413 → 369	-25	-10	-14	-23
PFOA[a]	413 → 169	-25	-10	-26	-9
PFTeDA	713 → 669	-45	-10	-20	-21
PFTeDA[a]	713 → 169	-45	-10	-36	-9
PFTrDA	663 → 619	-45	-10	-20	-19
PFTrDA[a]	663 → 169	-45	-10	-36	-17
PFUdA	563 → 519	-35	-10	-16	-17
PFUdA[a]	563 → 269	-35	-10	-26	-15

[a]Indicates a confirmatory ion.

[b]N-EtFOSSA = N-ethyl perfluorooctane sulfonamideo acetic acid; N-MeFOSAA = N-methyl perfluorooctane sulfonamideo acetic acid; PFBS = perfluorobutanesulfonic acid; PFDA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexanesulfonic acid; PFHxA = perfluorohexanoic acid; PFNA = perfluorononanoic acid; PFOS = perfluorooctanesulfonic acid; PFOA = perfluorooctanoic acid; PFTeDA = perfluorotetradecanoic acid; PFTrDA = perfluorotridecanoic acid; PFUdA = perfluoroundecanoic acid.

Table 3.2 Mass spectrometry parameters for PFAS internal standards

Analyte[a]	*m/z*	Declustering Potential (volts)	Entrance Potential (volts)	Collision Energy (volts)	Cell Exit Potential (volts)
PFTeDA-$^{13}C_2$	715 → 670	-50	-10	-20	-21
PFBS-$^{13}C_3$	302 → 99	-75	-10	-36	-11
PFHxS-$^{13}C_3$	402 → 99	-105	-10	-72	-11
PFHpA-$^{13}C_4$	367 → 322	-20	-10	-14	-19
PFHxA-$^{13}C_5$	318 → 273	-15	-10	-12	-15
PFDA-$^{13}C_6$	519 → 474	-35	-10	-16	-15
PFUdA-$^{13}C_7$	57 0→ 525	-75	-10	-16	-17
PFOA-$^{13}C_8$	421 → 376	-30	-10	-14	-19
PFOS-$^{13}C_8$	507 → 99	-115	-10	-96	-11
PFNA-$^{13}C_9$	472 → 427	-30	-10	-16	-13
PFBA-$^{13}C_4$	217 → 172	-10	-10	-14	-19
PFDoA-$^{13}C_2$	615 → 570	-200	-10	-38	-19
PFTeDA-$^{13}C_2$	715 → 670	-50	-10	-20	-21

[a]PFTeDA-$^{13}C_2$ = perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid; PFBS-$^{13}C_3$ = sodium perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate; PFHxS-$^{13}C_3$ = sodium perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate; PFHpA-$^{13}C_4$ = perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid; PFHxA-$^{13}C_5$ = perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid; PFDA-$^{13}C_6$ = perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid; PFUdA-$^{13}C_7$ = perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid; PFOA-$^{13}C_8$ = perfluoro-n-[$^{13}C_8$]octanoic acid; PFOS-$^{13}C_8$ = sodium perfluoro-[$^{13}C_8$]octanesulfonate; PFNA-$^{13}C_9$ = perfluoro-n-[$^{13}C_9$]nonanoic acid; PFBA-$^{13}C_4$ = perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid; PFDoA-$^{13}C_2$ = perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid.

1. Brookings, SD #1
2. Brookings, SD #2
3. Volga, SD
4. Marshall, MN
5. Minneapolis, MN #1
6. Minneapolis, MN #2
7. Iowa City, IA #1
8. Iowa City, IA #2
9. Iowa City, IA #3
10. Carson, CA
11. Oklahoma City, OK
12. Huntington, WV
13. Culloden, WV
14. Fairfield, NJ

Figure 3.1 Geographical distribution of drinking water samples obtained from across the United States. The blue pins indicate the town/city where the drinking water was sampled.

4.2.5 Method validation

The ability to differentiate PFAS from other interferents in drinking water was determined by extraction and analysis of blank drinking water and drinking water fortified with PFAS at the LLOQ concentrations (0.5 and 1.0 ng/L). Selectivity was evaluated by comparing the signal of PFAS from the fortified drinking water to the signal produced by the blank drinking water at the retention time of each PFAS analyte. Absence of compounds eluting in the blank water at the PFAS retention times was used to confirm the selectivity of the method. Calibration curves were constructed for PFAS from 0.5 to 500 ng/L in drinking water. Calibration and quality control (QC) standards were prepared by fortifying drinking water with 100 µL of the appropriate PFAS concentration as describe above. The calibrators were extracted and analyzed by the method presented here. The peak areas for each analyte and its respective internal standard were calculated by integration from baseline to baseline in MultiQuant software. The average peak area signal ratios of each analyte to its internal standard were plotted as a function of concentration. Both weighted ($1/x$ and $1/x^2$) and non-weighted linear least squares techniques were used to fit the calibrators and the most appropriate calibration range was found by visually inspecting residuals and evaluating each potential calibration curve's Percent Residual Accuracy (PRA).[60] The LLOQ was defined by investigating the above calibrators and determining the lowest concentration calibrator which satisfied the following criteria: (1) percent relative standard deviation (%RSD) of < 20% (to measure precision) and (2) percent accuracy within ± 20% back-calculated from the nominal concentration of each calibration standard. The ULOQ was similarly defined by the highest concentration tested which satisfied these criteria. The LOD was estimated by

analyzing concentrations below the LLOQ and was defined as the lowest analyte concentration that consistently produced a S/N ratio of 3 with noise measured as peak to peak in the blank noise, over the retention time of each compound.

Calibration curves were constructed on three separate days to evaluate the intra- and interassay performance of the method. Three QC standard concentrations for each PFAS compound were also prepared on the same days in drinking water (i.e., 1.0/10, 10/50, and 50/100 ng/L as low, medium, and high QCs, respectively). Two concentrations per level (low, medium, high) were necessary due to sensitivity differences among the PFAS. QC standards were analyzed in quintuplicate on three days (within seven calendar days). The accuracy and precision (%RSD) of the method was calculated by comparing back-calculated concentrations of quality control fortifications, including LLOQs, to the nominal concentrations of these standards. Intraassay accuracies and precisions were calculated from the QCs on each day's analysis and the interassay accuracies and precisions were determined by evaluation of the QCs over the entire three days. The symmetry of the chromatographic peak, as measured by peak asymmetry (A_s), was determined by dividing the front width by the back width at 10% peak height.

The matrix effect was determined by preparing calibration curves of PFAS spiked into drinking water and solvent (purified water) prepared as described and dividing the slopes to quantify the matrix effect (i.e., matrix/purified water). Non-equivalent slopes were interpreted as an indication of matrix effects. The recovery of each compound was determined from fortified drinking water at four different concentrations (10, 20, 50, and 100 ng/L). Recoveries of PFAS compounds were determined as a percentage by

comparing peak areas obtained from the fortified drinking water compared to standards prepared in purified water. All recovery experiments were performed in triplicate.

4.2.6 Comparison to SBSE

Due to the limited number of microextraction methods available in the literature for ultratrace analysis of PFAS and in an effort to evaluate the effectiveness of freeze concentration in ICECLES, ICECLES was compared to SBSE for PFAS-fortified purified water standards. Water was fortified at 10 ng/L and 100 ng/L in triplicate for each technique. Each technique was performed simultaneously using the same sample size, extraction time, back-extraction solvent, solvent volume, and desorption time, in an effort to accurately compare the signal difference between the two techniques. While the extraction time was longer than the typical SBSE extraction, direct comparison of the two techniques dictated extraction times equivalent to ICECLES.

When directly comparing ICECLES to SBSE for the analysis of PFAS from purified water (100 ng/L), ICECLES generated higher mean area counts (n = 3) for all 14 PFAS compounds tested. Specifically, ICELCES produced signals 1.1 (PFDA) to 216x (PFHxA) higher than those of SBSE. For a 10x dilution of PFAS (10 ng/L), ICECLES was capable of detecting all 14 compounds, while SBSE could only detect six compounds.

4.3 Results and discussion

4.3.1 Analysis of PFAS

The method presented here is a simple and sensitive technique for the ultratrace analysis of PFAS compounds from drinking water. The method features automated ICECLES extraction of PFAS directly from relatively small volumes (10 mL) of drinking

water, while using minimal organic solvent (i.e., 50 µL for the entire extraction). HPLC-MS/MS is used to analyze PFAS compounds from a 100 µL back-extract (i.e., methanol:water, 1:1 v:v) of the sorptive stir bar following ICECLES. Although a reusable sorptive stir bar is necessary, ICECLES precludes the need for single-use consumables (i.e., SPE cartridges) and the customized sorbent phases required for the SBSE and MMF-SPME methods listed in Table 4.3. An extremely small volume of methanol (50 µL) is the only reagent necessary for sample preparation; whereas, the vast majority of sample preparation techniques for PFAS compounds require many times more organic solvent for sample preparation. Specifically, the SPE selected methods in Table 4.3 require between 4 and 60 mL of organic solvent (methanol for most, but acetone, acetonitrile, and methanol for the Skutlarek et al.[85] method). This amount of organic solvent is 80 to 1200x more than the SPE-based methods and 10 to 168x less than the methods microextraction methods listed in Table 4.3. Additionally, ICECLES allows for a small sample volume (10 mL) versus 100-5000 mL samples (10 to 500x ICECLES) used in the SPE methods shown in Table 4.3.

Table 3.3 Comparison of extraction techniques for PFAS in drinking water.

Sample Preparation Technique[a]	# of PFAS analyzed	LLOQs for PFOA/PFOS (ng/L)	Accuracy (%)[b]	Precision (%)[b]	Validated Matrix[c]	Sample Volume (mL)	Organic Solvent	Organic Solvent Volume (mL)	Filtration needed?	Evaporation and reconstitution?	Automated?	Time (hr)[d]	Reference
SPE	15	NR[e]	<20 - >150 (not stated)	Not stated	Purified water	500	CH_3OH	4.0	Yes, 2x	Yes	Not stated	3.0	Domingo et al.[17]
SPE	11	NR	0 – 98 (n = 1)	Not stated	Drinking water	5000	CH_3OH	60	Yes	Yes	Not stated	14	Troger et al.[18]
SPE	17	0.3 / 0.01	71 -105 (not stated)	≤18 (not stated)	Purified water	250	CH_3OH	18.2	Yes	Yes	Not stated	3.0	Li et al.[19]
SPE	12	NR	11 – 117 (n = 4)	≤7.9 (n = 4)	Purified water	100	$(CH_3)_2CO$, CH_3CN, CH_3OH	10.1	No	Yes	Not stated	2.0	Skutlarek et al.[21]
SPE	21	2.8 / 1.3	NR[e] – 126 (n = 5)	≤28 (n = 5)	Purified water	5.0 / 250[f]	CH_3OH[f]	8.0	No	Yes	Not stated	5.0[e]	Llorca et al.[26]
SPE	13	NR	Not stated	Not stated	Not stated	500	CH_3OH	6.0	Yes, 2x	Yes	Not stated	3.0	Ericson et al.[22]
SPE	14	1.44 / 0.39	82 – 132 (n = 1)	≤26 (n = 1)	Purified water	1000	CH_3OH	9.0	Yes, 2x	Yes	Yes	5.5	Essumang et al.[23]
SPE	10	0.33 / 0.03	Not stated[g]	≤39[g] (not stated)	Not stated	500	CH_3OH	15.6	Yes, 2x	Yes	Not stated	3.0	Haug et al.[25]
SPE	7	0.5 / 0.66	79 – 150 (not stated)	Not stated	Purified water	1000	CH_3OH	9.0	Yes	Yes	Not stated	4.5	Thompson et al.[24]
MMF-SPME	6	1.32 / NR	80 – 119 (n = 4)	≤11 (n = 4)	Drinking water	20	CH_3CN, CH_3OH	0.5	Yes	Yes[h]	Yes	2.5	Huang, et al.[28]
SBSE	13	1.0 / 0.4	80 – 122 (n = 5)	≤12 (n = 5)	Not stated	10	CH_3OH	8.4	No	Yes[h]	Yes	1.5	Yao et al.[27]
ICECLES	14	0.5 / 0.5	73 – 116 (n = 15)	≤19 (n = 15)	Drinking water	10	CH_3OH	0.05	No	No	Yes	3.0	This work

[a] Stir bar sorptive extraction; Solid-phase extraction; Ice concentration linked with extractive stirrer.
[b]Accuracy and precision ranges across all analytes and concentrations tested.
[c]This reflects the matrix that was validated (if applicable). "Not stated" indicates the lack of information on whether a validation occurred and/or what matrix was used for validation.
[d]Analysis time accounts for the total sample preparation, SPE cleanup, evaporation and reconstitution (if applicable) time needed for one sample (does not account for instrument analysis). A common flow rate for SPE preparation (1 drop/s = 7 mL/min) was used to estimate the time necessary for SPE if they were not explicitly stated in the references. Estimates of the sample preparation time assume 1 hr for filtration and 1 hr for evaporation and reconstitution.
[g]NR = Not reported.
[f]Five mL was for the online SPE and 250 mL was used for offline SPE. Eight mL of methanol was used for offline SPE. Per the method, five hours was needed for offline SPE.
[g]No validation occurred in this paper; instead the authors referenced an external validation.
[h]Both the MMF-SPME and SBSE appear to utilize evaporation and reconstitution to generate higher concentration factors (i.e., evaporating 0.4 mL and reconstituting with 0.1 mL).

Figure 4.2 shows representative total ion chromatograms of PFAS (100 ng/L) extracted from a spiked drinking water sample and analyzed via the ICECLES-HPLC-MS/MS method. The HPLC-MS/MS runtime was 14 min, with PFAS compounds eluting between approximately 7.0 and 9.0 min. While drinking water can contain many interfering molecules, especially for highly sensitive methods, the high selectivity of the MS/MS instrument and mitigation of matrix effects via ICECLES produced complete resolution of all PFAS compounds from other components in drinking water. The excellent selectivity of the method was evident by the absence of co-eluting species in the blanks over the elution times of the 14 PFAS compounds. The peak shapes for all 14 PFAS compounds were sharp and generally symmetrical with peak asymmetry values ranging from 0.89 (i.e., slight fronting) to 2.4 (i.e., moderate tailing). The delay column and mobile phase gradient resulted in elution of the PFAS originating from the HPLC system between approximately 10.5 and 12.5 min, well past the elution times of the PFAS originating from the sample.

4.3.2 Method optimization

ICECLES utilizes relatively small sample volumes (10 mL) and concentrates analytes from the liquid phase into a sorptive stir bar. The entire freezing process is automated and only the stir speed is adjusted during ICECLES (i.e., a 10 mL sample is stirred at 1200 rpm for 2 to 2.5 hr, before the stirring speed is reduced to 300 rpm for approximately 30 more min). The desorption solvent for back-extraction post-ICECLES was optimized via several experiments with different solvents and solvent ratios. Two organic solvents commonly used for LC, acetonitrile and methanol, were evaluated. Methanol (100%) generated better peak shape and more signal than 100% acetonitrile for

all 14 PFAS tested. However, the peak shape for back extraction with either solvent at 100% was not optimal. Therefore, each back-extraction solvent was evaluated as a 50% mixture with purified water (i.e., a 1:1 organic solvent:water ratio). Again, methanol generated increased signals compared to acetonitrile, and with the addition of water, better peak shape was obtained (Figure 4.2). Therefore, methanol:water (1:1) was used as the desorption solvent for the remainder of this work.

Once the back-extraction solvent was determined, its volume was optimized with the intention of using the smallest volume which generated reproducible results. The purpose of using smaller back-extraction solvent volumes was to generate a greener method (i.e., reduction of organic solvent use) while simultaneously increasing the concentration factor of the extraction by reducing dilution of the back-extracted PFAS compounds. Four back-extract volumes were compared: 100, 200, 300, and 500 µL. The 100 µL back-extraction volume performed best, generating the highest signals for all 14 PFAS compounds while maintaining the reproducibility of the method. Therefore, all future experiments were carried out with this volume.

The effect of back-extraction time was also investigated. Back-extractions were performed for 5-30 min, with no discernable difference (i.e., signal output) observed between the desorption times tested. Therefore, a back-extraction times of 5 min was selected for the method.

In aggregate, the final optimized method included stir bar removal from the sample vial following ICECLES, placing into an HPLC vial insert containing 100 µL of methanol:water (1:1), back extracting for 5 min at room temperature with no

shaking/stirring, removal of the stir bar from the vial insert, and capping the vial prior to HPLC-MS/MS analysis.

4.3.3 LODs, LLOQs, and linear range

The ICECLES-HPLC-MS/MS method produced excellent sensitivity for the analysis of PFAS from drinking water. The LLOQs ranged from 0.5 to 1.0 ng/L and LODs ranged from 0.05 to 0.3 ng/L. A comparison of the PFAS LODs produced by the ICECLES-HPLC-MS/MS method to the methods listed in Table 4.3 can be found in Table 4.4. The ICECLES method produced LODs which compare favorably to these other ultratrace methods, while only requiring 10 mL of sample and an extremely small amount of organic solvent. Specifically, the Li et al. and Essumang et al. studies are the studies from Table 4.3 reporting consistently lower LODs than the ICECLES method, but these methods require 250 and 1000 mL of sample (25 and 100x the current method), 18.2 and 9.0 mL of organic solvent (364 and 180x the current method), evaporation and reconstitution, and the LODs reported are based on a purified water matrix instead of drinking water.

The dynamic range of the ICECLES-HPLC-MS/MS method was excellent. Calibration curves for the analysis of PFAS in drinking water were created in the range of 0.5 to 500 ng/L. Evaluation of multiple linear ranges and weighting factors revealed that the method produced a linear range from 0.5 to 500 ng/L using 1/x weighting. This linear range is large, spanning three orders-of-magnitude, and extends to the highest concentration tested. Once the linear range was established, calibration curves were prepared on three separate days to evaluate the stability of the calibration. Table 4.10 provides slope, intercept, R^2 and Percent Residual Accuracy (PRA)[60] values for all 14

PFAS over the course of the validation. While the R^2s and PRAs were consistent over the course of the three days (i.e., the R^2 and PRA values for the calibration curves were ≥0.98 and 83 to 98%, respectively), the calibration equations were not. For example, the reproducibility of the slopes was poor for most analytes, ranging from 5.1 to 27% relative standard deviation for those analytes with internal standards, and increasing up to 163% for those analytes without internal standard (i.e., N-EtFOSAA and N-MeFOSAA). Therefore, calibration curves should always be prepared on the day of analysis to ensure accurate concentrations are determined.

4.3.4 Accuracy, precision, matrix effect, and recovery

The ICECLES-HPLC-MS/MS method's accuracy and precision were evaluated at three concentrations in quintuplicate: 1.0/10, 10/50, and 50/100 ng/L over the course of three days (n = 15 for each concentration). Tables 4.5 – 4.7 shows the method accuracy and precision from this analysis. Considering 14 analytes are simultaneously quantified, the method generated satisfactory intraassay accuracy between 73 and 116% and good interassay accuracy between 87 – 108%. Aggregate intraassay precision was between 1.7 and 18% RSD and aggregate interassay precision was between 6.3 and 19% RSD. The comparison of solvent- and matrix-based calibration curves demonstrated a negligible matrix effect for PFAS in drinking water and evaluation of recovery at 10, 20, 50, and 100 ng/L for all compounds revealed excellent recovery (n = 3) for the method, ranging from 87 to 107, 80 to 103, 83 to 115, and 84 to 110% for these concentrations, respectively.

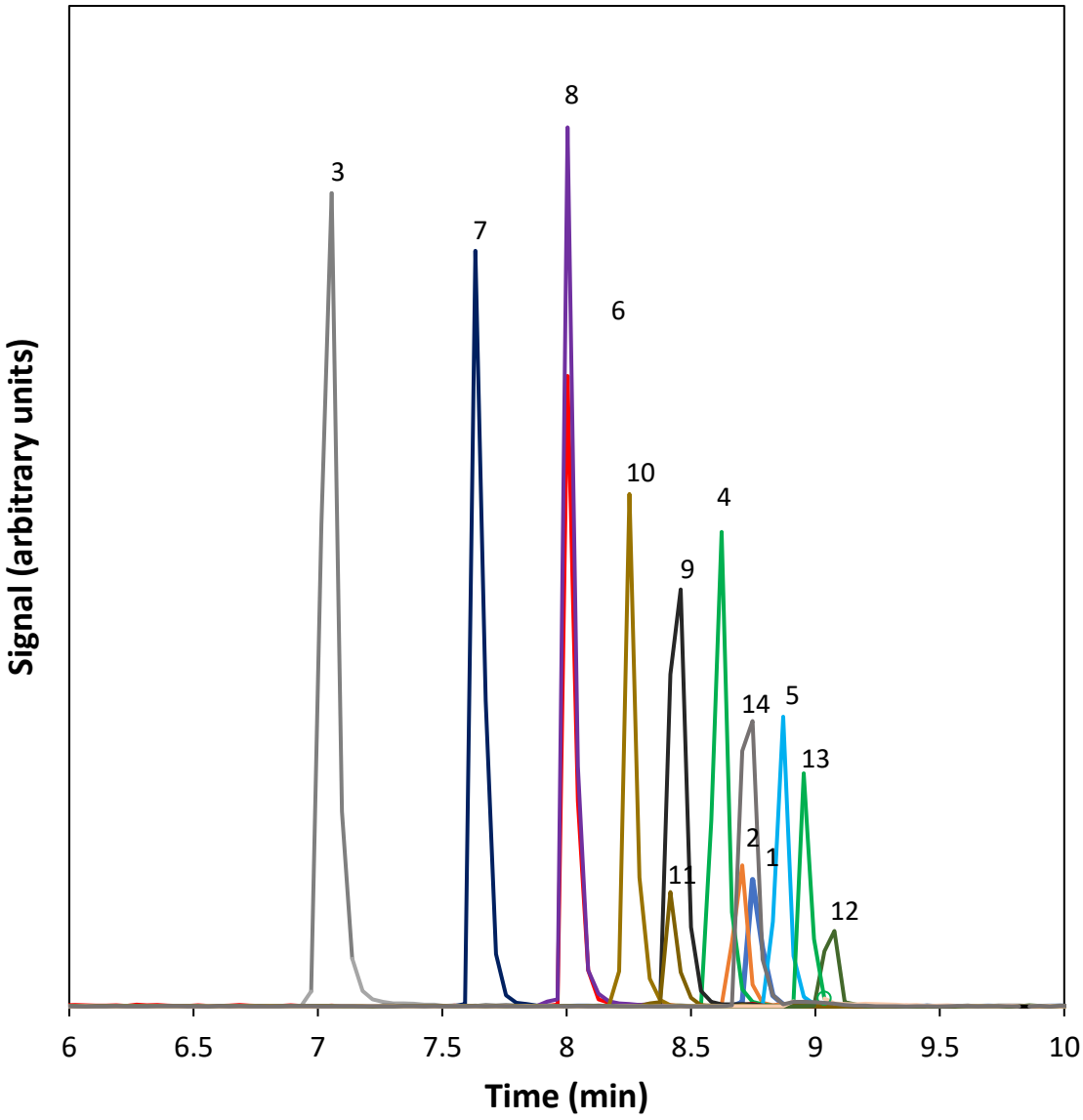


Figure 3.2 Total ion chromatogram of a drinking water blank sample fortified at 100 ng/L for 14 PFAS compounds. (1) N-EtFOSAA, (2) N-MeFOSAA, (3) PFBS, (4) PFDA, (5) PFDoA, (6) PFHpA, (7) PFHxA, (8) PFHxS, (9) PFNA, (10) PFOA, (11) PFOS, (12) PFTeDA, (13) PFTrDA, (14) PFUdA.

Table 3.4 Comparison of LODs[b] (ng/L) with other similar methods for PFAS analysis.

Study[a]	Technique	PFDA	N-EtFOSAA	N-MeFOSAA	PFDoA	PFHpA	PFHxA	PFNA	PFOA	PFTeDA	PFTrDA	PFUdA	PFBS	PFHxS	PFOS
Yao et al.[24]	SBSE-LC-MS/MS	0.13	--	--	0.23	0.27	0.34	0.17	0.40	--	--	0.21	--	0.06	0.10
Domingo et al.[14]	SPE-LC-MS/MS	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Troger et al.[15]	SPE-LC-MS/MS	0.0[c]	--	--	0.1	0.0[c]	0.1	0.0[c]	0.0[c]	--	--	0.0[c]	0.0[c]	0.0[c]	0.0[c]
Li et al.[16]	SPE-LC-MS/MS	0.06	--	--	0.04	0.04	0.02	0.02	0.09	0.14	0.07	0.08	0.03	0.01	0.0[c]
Skutlarek et al.[18]	SPE-LC-MS/MS	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Llorca et al.[23]	SPE-LC-MS/MS	2.4	0.50	--	3.5	5.1	7.4	1.9	0.83	3.2	10	1.2	2.5	0.27	0.39
Ericson et al.[19]	SPE-LC-MS/MS	0.12	--	--	--	--	0.17	0.15	0.85	0.06	--	0.07	0.07	0.02	0.12
Essumang et al.[20]	SPE-LC-MS/MS	0.03	--	--	--	0.02	0.01	0.03	0.43	0.05	0.01	0.79		0.01	0.39
Haug et al.[22]	SPE-LC-MS/MS	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Thompson et al.[21]	SPE-LC-MS/MS	0.12	--	--	--	0.15	0.13	0.15	0.13	--	--	0.13	0.14	0.18	0.13
This work	ICECLES-LC-MS/MS	0.10	0.3	0.3	0.3	0.15	0.10	0.08	0.08	0.15	0.15	0.15	0.08	0.05	0.10

[a]The Domingo et al.[14] and Haug et al.[22] studies did not state the LODs of their methods.
[b]Most techniques validated from purified water. See Table 14 for validation matrix.
[c]Reported as 0.0 in this study.

4.3.4 Application of the method

In order to determine the prevalence of PFAS in U.S. drinking water sources and to evaluate the validated ICECLES-HPLC-MS/MS method, drinking water samples from locations across the U.S. were analyzed (Figure 4.1). These included samples from urban and more rural areas, including Brookings and Volga (SD) Marshall and Minneapolis (MN), Iowa City (IA), Carson (CA), Huntington and Culloden (WV), Oklahoma City (OK), and Fairfield (NJ). For some locations, water was sampled from multiple point sources to give an indication as to if the PFAS originate from the drinking water source or from the point location (e.g., the building where the water is sampled). Figure 4.3 shows representative chromatograms of various PFAS compounds in the drinking water samples analyzed and Table 4.9 lists their PFAS concentrations found for each drinking water sample analyzed.

PFAS concentrations in the drinking water samples tested ranged from not-detected to 213 ng/L PFOA. These concentrations were in line with other published methods, which found concentrations between ND and 519 ng/L (PFOA from Skutlarek et al.).[85-87, 90] The profile of PFAS found in the water samples varied greatly. For instance, N-EtFOSAA, PFTeDA, and PFTrDA were not detected in any of the samples tested from this study, while PFOA was detected in all but two samples (86%). The most detected PFAS compounds in this work were PFOA (86%), PFHxA (80%), PFBS (71%), PFHpA (36%), PFOS (29%), and PFHxS (29%). These trends are similar to the relative abundance of PFAS in drinking water found by the other methods listed in Table 4.3. For instance, PFOA was one of the most detected compounds, detected in all but two studies.[86, 90] In general, previous studies found PFOA, PFOS, and PFHxS more often than

other PFAS compounds. Of the PFAS compounds detected in this study, the maximum concentration was 213 ng/L PFOA. Other PFAS detected at concentrations above 20 ng/L were PFHxA (40.4 ng/L), PFDA (36.0 ng/L), PFHpA (27.4 ng/L), and PFNA (23.3 ng/L). While PFBS was detected in 71% of all samples, the maximum concentration of PFBS was only 2.51 ng/L. Because PFBS has the lowest log K_{ow} (i.e., 1.8) of the PFAS detected, the ability of ICECLES to extract moderately hydrophilic compounds is most applicable to this compound. This may account for ICECLES detection of PFBS while the other microextraction techniques listed in Table 4.3 did not detect this compound. Additionally, when one PFAS is present in a water sample, it is commonly accompanied by other PFAS. There were two samples with no PFAS detected, and one sample with a single PFAS. All other samples had at least three PFAS present: 3 (n = 2), 4 (n = 5), 5 (n = 2), 7 (n= 1), and 8 (n = 1).

4.3.5 Comparison of ICECLES-HPLC-MS/MS to other ultratrace methods

Tables 4.3 and 4.4 list important parameters for the current technique and other selected techniques for ultratrace PFAS analysis from drinking water, including LODs, sample volumes, accuracy and precision values from validations and/or recovery experiments, organic solvent requirements, extraction times, and any additional steps needed (i.e., filtration, evaporation, reconstitution). (Note: While the ICECLES-HPLC-MS/MS method was validated in drinking water, most of the other techniques were validated in purified water, so a true comparison to some of the methods listed is not possible.) ICECLES was similar to the other methods listed in Table 4.3 in a number of ways, generating similar accuracy and precision, LODs, and analysis time requirement (i.e., approximately 3 hr; where the median extraction time for methods presented is 2.5

hr). For instance, the LODs for PFOA and PFOS for the Table 4.3 methods ranged from 0.08 – 2.0 ng/L and 0.01 – 2.0 ng/L, respectively. ICECLES generated LODs of 0.08 for PFOA and 0.10 ng/L for PFOS. Conversely, there are stark differences between the ICECLES method and the others listed in Table 4.3. The ICECLES method required much less sample volume than the SPE techniques (10 mL vs 100-5000 mL), 80-1,200x less organic solvent per sample, and was validated from drinking water. Furthermore, ICECLES does not require single-use consumables and is automated, without the need for continuous monitoring of SPE manifolds. ICECLES directly extracts drinking water without further cleanup (i.e., most other methods require filtration prior to extraction and evaporation, with reconstitution, following extraction).

Table 3.5 Intra- and interassay accuracy and precision of QCs based on 3 days of analysis.

Analyte	Concentration (ng/L)	Intraassay accuracy (%)[a]	Interassay accuracy (%)[b]	Intraassay precision (%RSD)[a]	Interassay precision (%RSD)[b]
PFDA	1.0	101 – 102	101	5.2 – 12	9.0
	10	87 – 104	97	3.4 – 6.9	9.9
	50	88 – 103	96	4.8 – 12	9.8
N-EtFOSAA	10	89 – 109	97	2.2 – 18	15
	50	83 – 108	95	8.9 – 17	16
	100	82 – 112	98	6.9 – 13	15
N-MeFOSAA	10	82 – 98	90	1.9 – 13	12
	50	85 – 109	94	7.0 – 13	15
	100	93 – 110	104	5.3 – 8.9	9.9
PFDoA	10	85 – 101	91	6.4 – 15	13
	50	81 – 97	87	11 – 12	14
	100	84 – 104	96	5.9 – 15	13
PFHpA	10	107 – 109	108	7.0 – 9.1	7.6
	50	94 – 115	105	2.8 – 12	11
	100	93 – 102	98	8.6 – 11	9.7

[a]Mean of the values for 1 day (n = 5 for each day); overall range for three separate days.
[b]Mean of the values over the three-day validation period (n = 15).

Table 3.6 Intra- and interassay accuracy and precision of QCs based on 3 days of analysis.

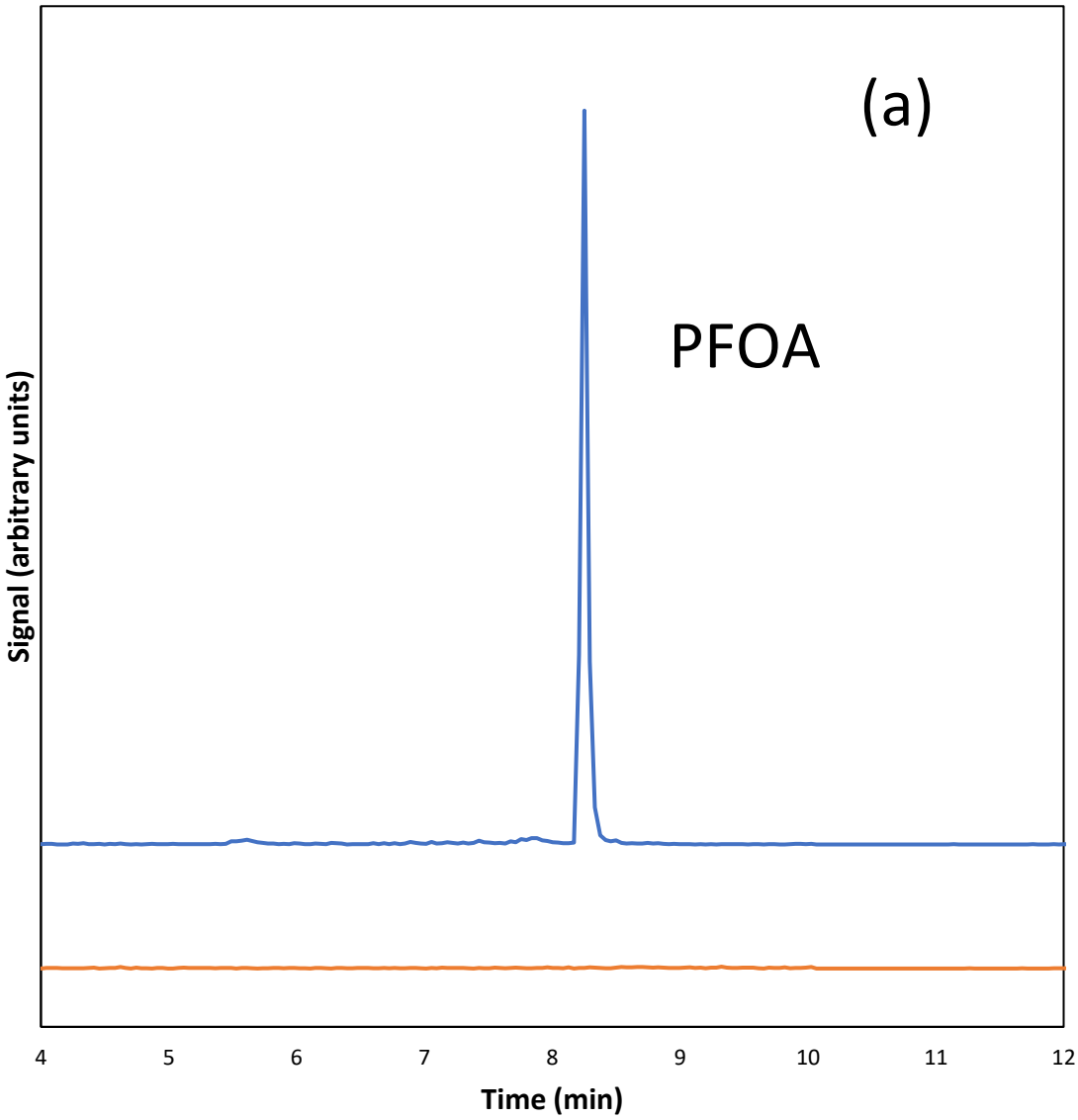
Analyte	Concentration (ng/L)	Intraassay accuracy (%)[a]	Interassay accuracy (%)[b]	Intraassay precision (%RSD)[a]	Interassay precision (%RSD)[b]
PFHxA	1.0	94 – 106	99	4.8 – 9.5	9.2
	10	100 – 112	107	4.3 – 15	10
	50	87 – 116	104	3.3 – 7.5	13
PFNA	1.0	103 – 110	105	3.7 – 9.8	8.0
	10	83 – 110	94	5.7 – 13	15
	50	88 – 115	97	2.4 – 15	15
PFOA	1.0	91 – 99	95	8.7 – 15	12
	10	97 – 104	100	8.1 – 12	9.8
	50	87 – 105	96	4.1 – 15	12
PFTeDA	10	90 – 107	97	6.1 – 13	12
	50	83 – 109	97	3.4 – 7.7	13
	100	92 – 109	102	5.2 – 9.9	10
PFTrDa	10	95 – 109	102	9.5 – 16	13
	50	73 – 100	91	1.7 – 15	19
	100	93 – 112	104	2.8 – 12	12

[a]Mean of the values for 1 day (n = 5 for each day); overall range for three separate days.
[b]Mean of the values over the three-day validation period (n = 15).

Table 3.7 Intra- and interassay accuracy and precision of QCs based on 3 days of analysis.

Analyte	Concentration (ng/L)	Intraassay accuracy (%)[a]	Interassay accuracy (%)[b]	Intraassay precision (%RSD)[a]	Interassay precision (%RSD)[b]
PFUdA	10	77 – 97	88	4.3 – 8.2	12
	50	85 – 95	88	3.0 – 7.8	7.5
	100	83 – 102	95	7.0 – 10	13
PFBS	1.0	94 – 107	101	4.3 – 8.4	7.8
	10	104 – 107	106	2.0 – 11	6.3
	50	100 – 102	101	3.3 – 11	6.4
PFHxS	1.0	87 – 104	98	8.3 – 13	13
	10	94 – 112	105	3.5 – 10	9.5
	50	88 – 109	101	5.4 – 8.2	11
PFOS	1.0	97 – 101	98	9.6 – 12	10
	10	102 – 111	106	5.5 – 11	8.1
	50	100 – 108	103	5.7 – 7.7	7.4

[a]Mean of the values for 1 day (n = 5 for each day); overall range for three separate days.
[b]Mean of the values over the three-day validation period (n = 15).


(a)
PFOA
Signal (arbitrary units)
4
5
6
7
8
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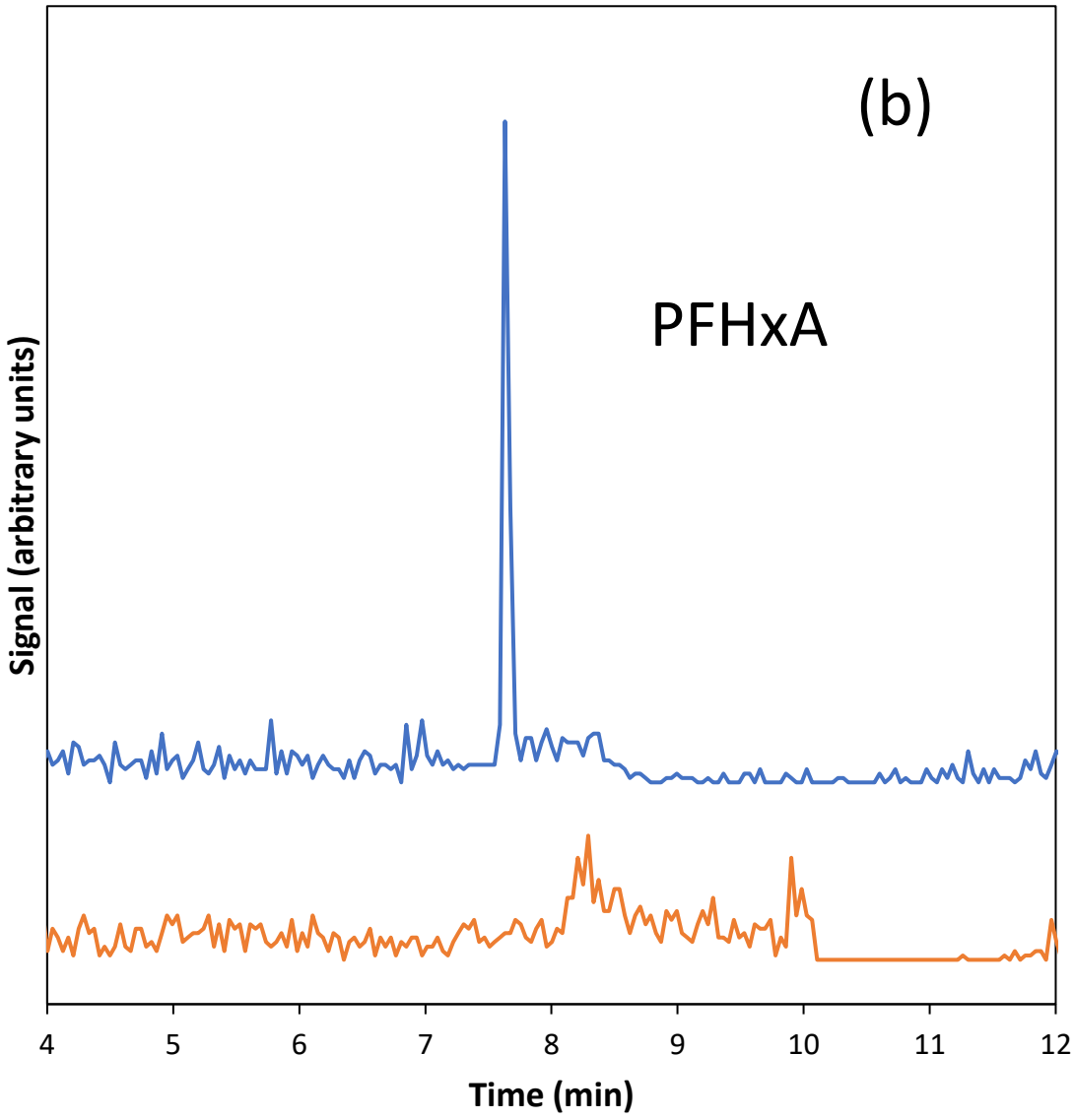
11
12
Time (min)


(b)
PFHxA
Signal (arbitrary units)
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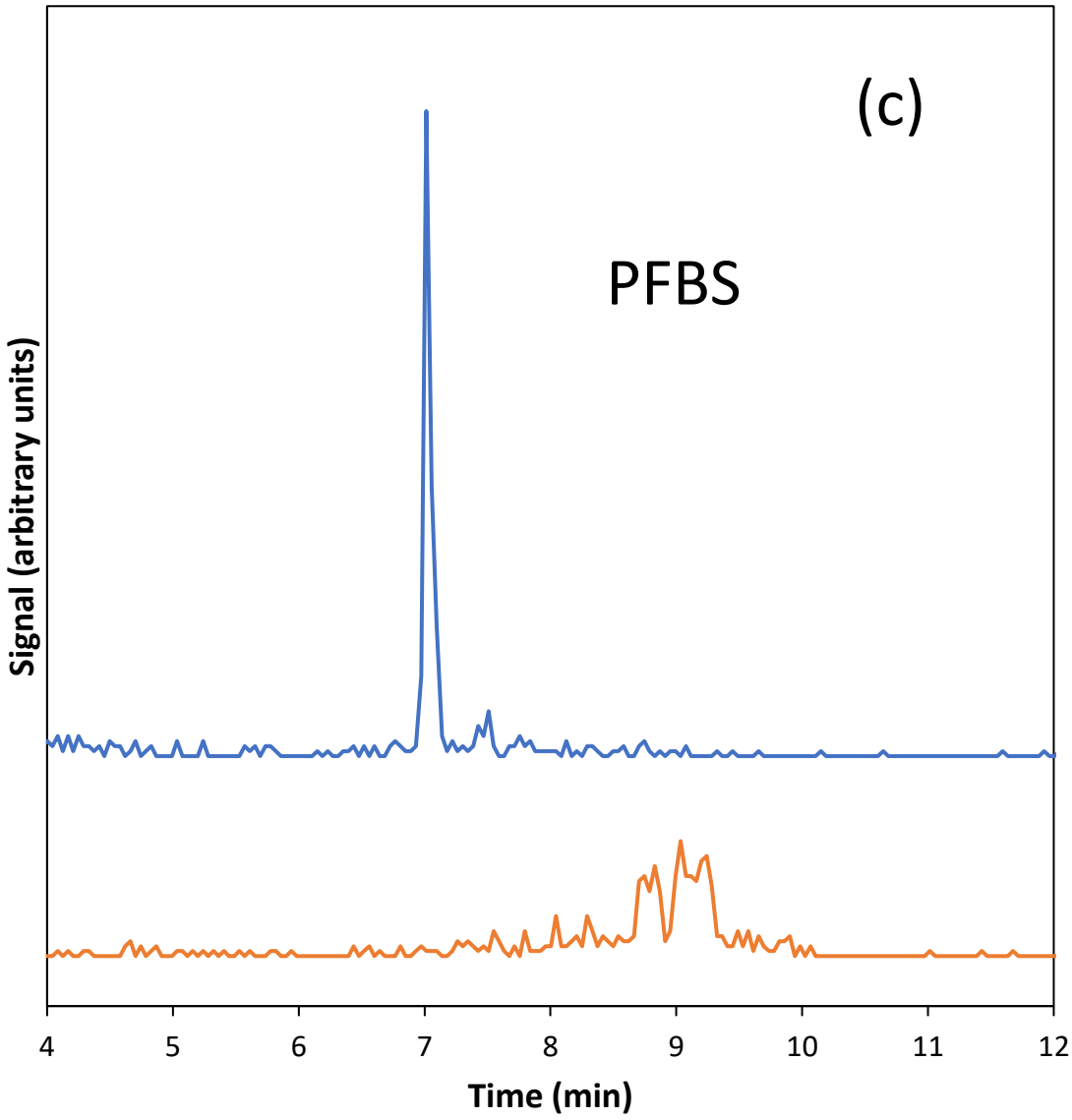
Time (min)


(c)
PFBS
Signal (arbitrary units)
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11
12
Time (min)

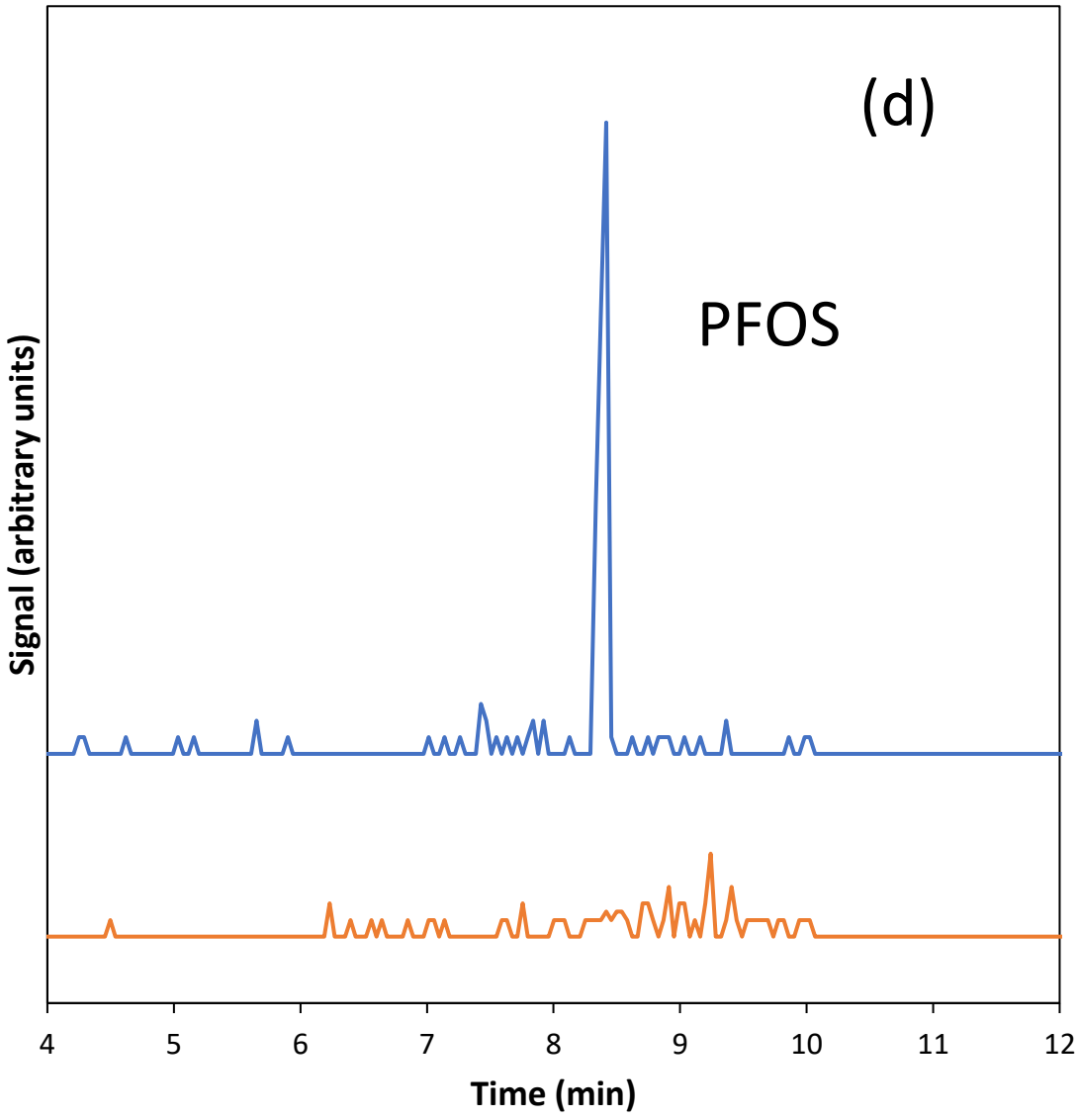


Figure 3.3 Various PFAS compounds in samples collected across the U.S. (blue) along with analysis of blank drinking water. (a) PFOA in Iowa hospital tap water, inset of PFOA in blank tap water, (b) PFHxA in Iowa City, IA residential tap water, (c) PFBS in Hunting, WV residential tap water, and (d) PFOS in SGS Fairfield (Fairfield, NJ) laboratory tap water.

Table 3.8 Field sample residues found (ng/L)

Sample ID	Sample #[a]	N-EtFOSAA	N-MeFOSAA	PFBS	PFDA	PFDoA	PFHpA	PFHxS
Brookings, SD #1	1	--	--	0.61	--	--	1.22	--
Brookings, SD #2	2	--	--	--	--	--	--	--
Volga, SD	3	--	--	1.80	--	--	--	--
Marshall, MN	4	--	--	1.81	--	--	--	0.60
Minneapolis, MN #1	5	--	--	1.14	--	--	--	0.71
Minneapolis, MN #2	6	--	--	--	--	--	--	--
Iowa City, IA #1	7	--	--	--	36.0	7.96	27.4	--
Iowa City, IA #2	8	--	1.38	--	1.76	--	1.18	--
Iowa City, IA #3	9	--	--	1.27	--	--	1.29	--
Carson, CA	10	--	--	0.63	--	--	--	--
Oklahoma City, OK	11							
Huntington, WV	12	--	--	2.51	--	--	--	(0.45)[a]
Culloden, WV	13	--	--	0.50	--	--	--	--
Fairfield, NJ	14	--	--	2.22	(0.35)	--	1.13	1.68

[a]Sample # is a cross-reference to Figure 4.1.

[b]Values in parentheses are estimated concentrations since they were below the LOQ but above the LOD.

Table 3.9 PFAS concentrations (ng/L) found in drinking water samples.

Sample ID	Sample #[a]	PFHxA	PFNA	PFOS	PFOA	PFTeDA	PFTrDA	PFUdA
Brookings, SD #1	1	--	--	--	--	--	--	--
Brookings, SD #2	2	--	--	--	--	--	--	--
Volga, SD	3	1.47	--	--	(0.40)[a]	--	--	--
Marshall, MN	4	0.50	--	--	(0.41)	--	--	--
Minneapolis, MN #1	5	0.70	--	--	2.48	--	--	--
Minneapolis, MN #2	6	--	--	--	1.66	--	--	--
Iowa City, IA #1	7	40.4	23.3	--	213	--	--	0.54
Iowa City, IA #2	8	1.79	--	--	8.23	--	--	--
Iowa City, IA #3	9	1.78	--	--	2.15	--	--	--
Carson, CA	10	2.24	--	0.87	0.90	--	--	--
Oklahoma City, OK	11	0.85	--	--	4.91	--	--	--
Huntington, WV	12	1.18	--	(0.44)[a]	4.43	--	--	--
Culloden, WV	13	0.53	--	1.70	0.67	--	--	--
Fairfield, NJ	14	2.47	(0.45)	5.62	6.83	--	--	--

[a]Sample # is a cross-reference to Figure 4.1.
[b]Values in parentheses are estimated concentrations since they were below the LOQ but above the LOD.

Table 3.10. Regression data for interassay validations of PFAS in drinking water.

Analyte	Day	Slope ($x10^{-3}$)	Intercept ($x10^{-3}$)	R^2	PRA[a]
PFDA	1	8.40	0.11	0.999	88
	2	8.22	0.26	0.997	88
	3	6.17	0.98	0.999	98
N-EtFOSAA	1	1.08	-0.29	0.998	91
	2	26.4	-0.28	0.990	94
	3	0.66	0.11	0.983	96
N-MeFOSAA	1	1.23	0.71	0.998	90
	2	58.5	-0.51	0.994	97
	3	1.23	0.85	0.995	84
PFDoA	1	5.09	5.73	0.999	96
	2	7.34	-56.5	0.992	92
	3	6.57	-9.85	0.999	88
PFHpA	1	10.6	1.44	0.999	91
	2	6.00	1.46	0.998	94
	3	9.28	4.68	0.998	90
PFHxA	1	9.11	3.85	0.995	92
	2	6.84	2.43	0.995	89
	3	8.17	3.47	0.999	96
PFNA	1	9.60	6.55	0.997	86
	2	8.29	18.7	0.996	90
	3	5.81	16.7	0.999	93
PFOA	1	7.51	3.56	0.999	93
	2	10.5	3.74	0.999	90
	3	8.90	5.01	0.997	90
PFTeDA	1	7.27	10.9	0.998	95
	2	8.59	21.4	0.997	90
	3	8.33	1.35	0.999	95
PFTrDa	1	13.4	14.5	0.999	91
	2	21.1	-45.7	0.980	83
	3	14.9	-25.5	0.997	89
PFUdA	1	8.22	25.2	0.990	88
	2	10.7	10.9	0.999	94
	3	8.19	12.4	0.998	92
PFBS	1	17.4	1.58	0.999	95
	2	15.9	5.10	0.998	90
	3	16.0	1.52	0.999	95
PFHxS	1	38.0	2.28	0.998	92
	2	33.1	18.7	0.999	95
	3	30.5	12.4	0.999	95
PFOS	1	8.44	2.55	0.998	86
	2	5.53	3.51	0.998	89
	3	7.18	1.63	0.998	93

[a]Percent Residual Accuracy

4.3.5 Conclusion

As the public becomes more concerned about what is in the drinking water supply, analytical techniques to detect PFAS at ultratrace concentrations are becoming increasingly necessary. The ICECLES-HPLC-MS/MS method developed here was able to generate LODs and LLOQs similar to other published methods while requiring relatively small sample volumes, small volumes of organic solvents, and no single-use consumables. This ICECLES-based extraction technique provided an accurate and precise analysis of 14 PFAS compounds in drinking water at ultratrace levels. Using this method, these compounds were detected and quantified in various field samples from across the U.S., with values ranging from ND to 213 ng/L (PFOA). At least one PFAS was found in the overwhelming majority of the drinking water samples tested, with most containing 3 or more PFAS. ICECLES is an alternative to current PFAS analysis methods, predominantly SPE-based techniques, offering an automated and greener extraction (only 50 µL of methanol per sample) for drinking water. In the future, the ruggedness of ICECLES-HPLC-MS/MS analysis of PFAS should be tested by analyzing other forms of water for PFAS contamination, including waste (influent and effluent), surface and ground water

Chapter 5. Development and validation of an HPLC-MS/MS method for fentanyl, hydrocodone, and codeine using direction injection

5.1 Introduction

Active pharmaceutical ingredients (APIs) have relatively recently been recognized as environmental contaminants in drinking water, generating concern over their environmental impact, as well as their consequences for human health. One class of API, opioids, have been recognized for their overuse in the medical community[95, 96] and their role in the illicit drug trade.[97] Opioids can be natural or synthetic, with compounds such as morphine and codeine occurring naturally (i.e., morphine and codeine are purified from opium poppies) and hydrocodone and fentanyl being synthetic. Modern opioid use arguably began in 1805, when Austrian pharmacist Friedrich Serturner successfully isolated and extracted morphine crystals from poppy seed juice.[34] In 1832, codeine was first isolated from opium poppies and has become the most widely used opioid in the world,[35] with approximately 360,000 kg produced every year. Similar to codeine, hydrocodone has become a widely used opioid, especially in the U.S. Fentanyl was discovered by Paul Jannsen in 1960 when searching for more effective pain medications. Fentanyl is a synthetic opioid that was approved for medical use by the U.S. FDA in 1968 and is now characterized by its highly addictive nature and negative impact on human health, causing over 20,000 overdose deaths in the U.S. in 2016 alone.[37, 38]

Due to their popularity, opioids have been increasingly detected in waterways, specifically surface and wastewater.[98-105] Their presence in U.S. water systems is generally attributable to human waste after medical or illicit use. There have also been instances of improper laboratory disposal into water sources.[106, 107] Studies have also

shown that once opioids contaminate water sources, conventional wastewater treatment practices may be incapable of efficient removal of these substances.[108-110] This has led to multiple opioids being detected in drinking water sources.[111-114] Table 5.1 provides a list of studies which analyzed drinking water for opioids. As seen in Table 5.1, multiple opioids have been found in drinking water for each previous study, except for the first to investigate this issue in 2006 (i.e., Hummel et al.[102]). The inability to detect quantifiable levels of opioids in drinking water by Hummel et al.[102] may be due to the relatively high limits of quantification of the method compared to later studies (Table 5.1) and the emerging nature of the problem in 2006. Evaluation of opioids in drinking water have detected many different opioids: morphine, codeine, norcodeine, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), fentanyl, methadone, dihydrocodeine, tramadol, ketamine, and oxycodone. These opioids were detected up to 76 ng/L (codeine),[115] with concentrations ranging from low to mid part-per-trillion (ng/L) levels. Although these opioid concentrations are considered ultratrace, they are still concerning, since the chronic health effects of continuous exposure to low concentrations of opioids are unknown.[116, 117] While the presence of opioids in water sources has been recognized as a potential problem since 2006, and the first study detecting opioids in drinking water was published in 2009, there is a paucity of studies reporting opioid concentrations in drinking water, and no study evaluating the concentration of opioids in U.S. drinking water sources.

Because there is minimal information concerning the presence of the opioids of concern in U.S. drinking water sources, the objective of this study was to develop a method for the ultratrace determination of fentanyl, hydrocodone, and codeine in

drinking water and to use this method to evaluate the prevalence of these opioids in drinking water supplies across the U.S.

5.2 Experimental

The following sections describe the materials and methods used for the analysis of opioids from drinking water using DI-HPLC-MS/MS.

5.2.1 Materials and standards

The reference standards (fentanyl, hydrocodone, and codeine; 1 mL of 1.0 mg/mL each in methanol) and internal standards (fentanyl-d_5, hydrocodone-d_3, and codeine-d_3; 1 mL of 100 µg/mL each in methanol) were purchased from Sigma Aldrich (St. Louis, MO). Acetonitrile (LC-MS grade), ammonium formate (LC-MS grade) and formic acid (LC-MS grade) were purchased from Fisher Scientific (Waltham, MA). A MilliQ® Ultrapure water system (MilliporeSigma) was used to generate purified water.

From the purchased stock standards, intermediate standard solutions were prepared in methanol at 10 mg/L and 100 µg/L, by 1:100 serial dilutions. From these intermediate standard solutions, working solutions (0.25 to 100 ng/L) were prepared in methanol. The internal standards were similarly diluted to the final concentration of 1 µg/L in methanol.

5.2.2 Drinking water samples

Drinking water for method development was obtained from the GLP residue laboratory taps at SGS North America, Inc. in Brookings, South Dakota. The drinking water samples were pre-screened for fentanyl, hydrocodone, and codeine prior to using them as blank material for fortifications. Drinking water samples that produced undetectable levels of opioids were used for method development and validation. None of

the opioids analyzed were detected in the laboratory tap water; therefore, this tap water source was used for method development, validation, quality controls, and calibration. In order to evaluate the potential for compounds leaching into the method development drinking water, a sample of this water was stored in a 1-L HDPE bottle for more than four months and then tested for these three opioids.

Water samples for analysis via the validated method were collected in multiple locations across the U.S. (Figure 5.1) via a standard sampling SOP. Specifically, each sample was collected from a tap by allowing cold drinking water to run freely at maximum flow for at least 3 min, a 1-L HDPE bottle was filled with tap water and the rinse was discarded, the HDPE bottle was again filled with tap water, the bottle was capped tightly, and the sample was shipped to the laboratory at SGS in Brookings, SD. Upon receipt, samples were stored in a refrigerator until analysis.

5.2.3 Fortification procedure and extraction

Drinking water (10 mL) was transferred into a 15-mL centrifuge tube and fortified by adding 100 µL of the working standard. Additionally, internal standard (100 µL of 1 µg/L) was added to each sample, standard, and blank resulting in a final internal standard concentration of 10 ng/L. The sample was then mixed briefly and allowed to sit at room temperature for 10 min. Next, an aliquot of the 10 mL sample was syringe filtered with a 0.22 µm polytetrafluoroethylene (PTFE) filter and transferred into a 2-mL HPLC vial for analysis.

1. Brookings, SD #1
2. Brookings, SD #2
3. Volga, SD
4. Marshall, MN
5. Minneapolis, MN #1
6. Minneapolis, MN #2
7. Iowa City, IA #1
8. Iowa City, IA #2
9. Iowa City, IA #3
10. Carson, CA
11. Oklahoma City, OK
12. Huntington, WV
13. Culloden, WV
14. Fairfield, NJ

Figure 4.1 Geographical distribution of drinking water samples obtained from across the United States.

Table 4.1 Prevalence of opioids in drinking water.

Opioids Analyzed	Opioids Detected	Prevalence	Concentration Range (ng/L)	Sample Locations	Method	LOQs (ng/L)	Reference
Benzoylecgonine Codeine Dihydrocodeine Hydrocodone Methadone Morphine Oxycodone Tramadol	--	--	--	Germany	SPE-LC-MS/MS[a]	5.0	Hummel et al., 2006[12]
Heroin 6-acetylmorphine Morphine Codeine Normorphine Norcodeine Fentanyl Methadone EDDP[b]	Morphine Codeine Norcodeine EDDP Fentanyl Methadone	18 of 18 (100%) 18 of 18 (100%) 10 of 18 (56%) 18 of 18 (100%) 1 of 18 (5.6%) 18 of 18 (100%)	1.5 – 12 14 – 76 <LOD – 7.9 5.2 – 31 <LOD – 8.5 1.9 – 9.4	Spain	SPE-LC-MS/MS	0.13 – 13[c]	Boleda et al., 2009[25]
Morphine Morphine glucuronide Normorphine Codeine Norcodeine Heroin 6-acetylmorphine Methadone EDDP Fentanyl	Methadone EDDP	5 of 26 (19%) 15 of 26 (58%)	0.2[d] 0.4[d]	Latin America[e] Japan Europe[f]	SPE-LC-MS/MS	0.1 - 12	Boleda et al., 2011[22]

Table 4.2 (continued) Prevalence of opioids in drinking water.

Opioids Analyzed	Opioids Detected	Prevalence	Concentration Range (ng/L)	Sample Locations	Method	LOQs (ng/L)	Reference
Morphine Morphine glucuronide Normorphine Codeine Norcodeine Heroin 6-acetylmorphine Methadone EDDP Fentanyl	Methadone EDDP Fentanyl	9 of 50 (18%) 43 of 50 (86%) 1 of 50 (2%)	<LOQ – 2.7 0.1 – 3.5 <LOQ – 1.4	Spain	SPE-LC-MS/MS	0.1 - 12	Boleda et al., 2011[22]
Codeine Acetylcodeine Dihydrocodeine Morphine Acetylmorphine Methadone Heroin Tramadol Ketamine Oxycodone EDDP Fentanyl	Codeine Dihydrocodeine Morphine Methadone Tramadol Ketamine Oxycodone Fentanyl	Not stated Not stated Not stated Not stated Not stated Not stated Not stated 3 of 3 (100%)	44[d] 5.7[d] 6.4[d] <LOQ[d] 5.4[d] 15[d] 5.1[d] 12[d]	Canada	POCIS[g]-LC-MS/MS	0.36 - 56	Rodayan et al., 2015[23]
Heroin Morphine 6-acetylmorphine Methadone EDDP[b]	Methadone EDDP[b]	4 of 28 (14%) 2 of 28 (7%)	<LOQ – 0.31 <LOQ – 1.41	Spain	Online-SPE-LC-MS/MS	0.04 – 1.26	Mendoza et al., 2016[24]
Codeine Hydrocodone Fentanyl	Codeine Hydrocodone	11 of 14 (79%) 11 of 14 (79%)	<LOQ – 20 <LOQ – 2.8	United States	DI[h]-LC-MS/MS	0.25 – 1.5	This work

[a]SPE = Solid phase extraction; LC-MS/MS = Liquid chromatography mass spectrometry mass spectrometry.
[b]EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.
[c]LOQs were not provided; therefore, the LODs provided were multiplied by 3.33 to estimate LOQs for this table.
[d]A complete range was not provided; therefore, the mean values are shown here.
[e]Latin America for this study refers to Argentina, Brazil, Chile, Colombia, Panama, Peru, and Uruguay.
[f]Europe for this study refers to Austria, France, Germany, Iceland, Slovakia, Switzerland, and United Kingdom.
[g]POCIS = Polar organic chemical integrative sampler. POCIS is a passive sampling device which collects water-soluble contaminants from water sources by capturing them on a sorbent material sandwiched between microporous membranes. The collection time typically can last days, weeks, or months.
[h]DI = Direct injection.

5.2.4 Liquid chromatography—tandem mass spectrometry

Analysis of opioids from tap water was carried out by a Shimadzu Nexera XR HPLC (Tokyo, Japan) and a Sciex 6500+ MS/MS (Redwood City, CA). An Agilent Poroshell C18 column (4.6 x 50 mm, 2.7 µm), 0.2% formic acid in 50 mM aqueous ammonium formate (Mobile Phase A), and 100% acetonitrile (Mobile Phase B) were used to separate the opioids. The mobile phase composition was initially set to 30% B, linearly increased to 50% B over 3.0 min, held for 2.0 min, decreased back to 30% B over 1.0 min, and then held constant for 1.0 min (7.0 min total run time). The flow rate was 0.6 mL/min and the column and autosampler temperatures were 40 °C and 10 °C, respectively. An injection volume of 75 µL was used with 500 µL of an autosampler rinsing solution (acetonitrile:IPA:methanol (1:1:1)) used before and after aspiration. The MS/MS was operated at 600 °C, with an Ion Spray voltage of 5500 V, ion source gas 1 of 65 psi, ion source gas 2 of 65 psi, curtain gas at 25 psi, and collision gas at 9 psi. The various mass spectrometry parameters for fentanyl, hydrocodone, codeine, and their internal standards are reported in Tables 5.2 and 5.3.

Table 4.3 Mass spectrometry parameters for opioids

Analyte	*m/z*	Declustering Potential (volts)	Entrance Potential (volts)	Collision Energy (volts)	Cell Exit Potential (volts)
Fentanyl	337→188	80	10	29	14
Fentanyl[1]	337→105	80	10	51	10
Hydrocodone	300→199	80	10	39	14
Hydrocodone[1]	300→128	80	10	73	14
Codeine	300→152	80	10	81	10
Codeine[1]	300→115	80	10	89	14

[1]Indicates a confirmatory ion.

Table 4.4 Mass spectrometry parameters for opioid internal standards

Analyte	*m/z*	Declustering Potential (volts)	Entrance Potential (volts)	Collision Energy (volts)	Cell Exit Potential (volts)
Fentanyl-d_5	342→188	91	10	33	12
Hydrocodone-d_3	303→199	71	10	41	8.0
Codeine-d_3	303→165	46	10	57	8.0

5.2.5 Method validation

The ability to differentiate opioids from other interferents in drinking water was determined by extraction and analysis of blank drinking water and drinking water fortified with opioids. Selectivity was evaluated by comparing the signal of the opioids from the fortified drinking water to the signal produced by the blank drinking water at the retention time of each opioid. The selectivity was further evaluated by calculating the resolution of the peaks corresponding to each analyte from the nearest peak which consistently produced detectable signals ($S/N \geq 3$).

Calibration curves were constructed for the opioids from 0.25 to 100 ng/L (0.25, 0.5, 1.0, 2.5, 5.0, 10, 25, 50, and 100 ng/L) in drinking water over multiple days (i.e., three separate days within seven calendar days). Specifically, calibration standards were prepared in triplicate by fortifying drinking water and analyzing by the method presented here. The peak areas for all three opioids and their respective internal standards were calculated by integration from baseline to baseline in MultiQuant™ software. The average peak area signal ratios of fentanyl to fentanyl-d_5, hydrocodone to hydrocodone-d_3, and codeine to codeine-d_3 were plotted as a function of concentration. The calibration curves were fit with both weighted ($1/x$ and $1/x^2$) and non-weighted least squares techniques and a $1/x$ weighting was chosen based on visual inspection of residuals and evaluation of the Percent Residual Accuracies [60] for multiple calibration curves range

(i.e., numerous combinations of dynamic ranges and weighting factors were evaluated to find the most appropriate linear range).

Three QC standard concentrations were prepared in drinking water (0.5/5.0, 5.0/25, and 25/50 ng/L as low, medium, and high QCs, respectively). The use of two concentrations per QC (low, medium, high) was necessary due to sensitivity differences among the opioids (i.e., the method is more sensitive for fentanyl than hydrocodone and codeine). QCs were prepared in the same fashion as the calibrators. The QC standards were not included in the calibration curve but were used to estimate the accuracy and precision (%RSD) of the method by back-calculating the estimated concentration of the QCs based on the calibration curve and determining the %RSD and accuracy at each QC concentration versus the true (i.e., nominal) QC concentration. The QC standards were analyzed in quintuplicate on the three days (within seven calendar days) that the calibration curves were constructed. Intraassay accuracies and precisions were calculated from the QCs on each day and the interassay accuracies and precisions were determined by evaluation of the QCs over the entire three days. The limits of quantification were defined by investigating the above calibrators and determining the lowest (i.e., lower limit of quantification (LLOQ)) and highest concentration (i.e. upper limit of quantification (ULOQ)) concentration calibrators which satisfied the following criteria: (1) percent relative standard deviation (%RSD) of < 10% (to measure precision) and (2) percent accuracy within ± 20% back-calculated from the nominal concentration of each calibration standard. The limit of detection (LOD) was estimated by analyzing concentrations below the LLOQ and was defined as the lowest analyte concentration that consistently produced a S/N ratio of 3, with noise measured as peak to peak in the blank

noise over the retention times of the opioids. The symmetry of the chromatographic peaks was quantified by peak asymmetry (A_s), which is calculated by dividing the front width by the back width at 10% peak height.

The matrix effect was determined by creating calibration curves of drinking water and purified water spiked with opioids and prepared as described. The calibration curves were then fit as described above and the slopes were compared. The matrix effect was quantified by calculating the ratio of calibration curve slopes (i.e., $m_{\text{drinking water}}/m_{\text{purified water}}$). Non-equivalent slopes were interpreted as an indication of matrix effects. Method recovery for these opioids was determined from fortified drinking water and purified water samples at two concentrations: 2.5 and 10 ng/L. Recoveries of fentanyl, hydrocodone, and codeine were analyzed to determine if the opioids were sorbing to the filter material. Recovery was quantified as a percentage by comparing peak areas obtained from the fortified drinking water with fortified solvent samples at the same concentrations. Recovery experiments were performed in quintuplicate.

5.3 Results and discussion

5.3.1 Analysis of opioids

The method presented here is a simple and sensitive technique for the ultratrace analysis of fentanyl, hydrocodone, and codeine from drinking water. The method features no sample preparation, aside from simple filtration, followed by injection and analysis via HPLC-MS/MS. This method eliminates the need for organic solvents during sample preparation, costly single-use consumables (e.g., SPE cartridges), specialized equipment or glassware, and time-consuming sample preparation steps (e.g.,

evaporation). The method does not require any organic solvent, while the other methods listed in Table 5.1 require anywhere from 1.0 to 100.4 mL per sample.

Figure 5.2 shows a representative chromatogram of each opioid extracted from a fortified drinking water sample and blank analyzed via the DI-HPLC-MS/MS method. The figure shows the quantification and identification MRM transitions for each analyte. The HPLC-MS/MS runtime was 7.0 min, with retention times for fentanyl, hydrocodone, and codeine of approximately 3.4, 1.4, and 1.3 min, respectively. The peak shapes for fentanyl, hydrocodone, and codeine were sharp and generally symmetrical with peak asymmetry values of 1.8, 1.6, and 0.9, respectively. The selectivity of the method was excellent, as evident by the absence of co-eluting species in the blanks over the retention times of the opioids.

5.3.2 DI-HPLC-MS/MS method performance

Considering the minimal sample preparation required, the DI-HPLC-MS/MS method produced excellent sensitivity for the analysis of fentanyl, hydrocodone, and codeine from drinking water. The LODs were 0.08, 0.15, and 0.5 ng/L and the LLOQs were 0.25, 0.5, and 1.5 ng/L for fentanyl, hydrocodone, and codeine, respectively. These values are similar or better than other methods[113, 115] for fentanyl and codeine analysis from drinking water, which produced LODs of 0.81 and 0.1, and 0.69 and 0.2 ng/L, respectively (note that hydrocodone wasn't included in these methods).

Calibration curves for the analysis of opioids in drinking water were evaluated in the range of 0.25 to 500 ng/L. Evaluation of multiple potential linear ranges and weighting factors revealed that the linear range was 0.25 to 100 ng/L using 1/x weighting. Linearity for the analysis of fentanyl, hydrocodone, and codeine was excellent, with R^2

values of ≥0.996 and PRA values for each calibration curve between 94-96, 90-95, and 92-94%, respectively. Once the linear range was established, calibration curves were prepared on three separate days to evaluate the calibration stability of the method. The calibration curves were highly stable over the course of the three days, producing consistent slopes, intercepts, R^2 values, and PRAs (Table 5.4).

Table 4.5 Regression data for interassay validation of opioids in drinking water.

Analyte	Day	Slope[a]	Intercept	R^2	PRA[b]
Fentanyl	1	0.9579	0.1220	0.9999	96
	2	0.9695	0.4045	0.9971	94
	3	0.9959	0.0881	0.9996	94
Hydrocodone	1	0.9888	0.5045	0.9960	95
	2	1.0112	-0.2424	0.9994	94
	3	1.0178	-0.4309	0.9985	90
Codeine	1	1.0032	-0.0792	0.9991	94
	2	1.0725	-0.8223	0.9993	92
	3	1.0179	-0.4352	0.9962	93

[a]Utilizes ratio of analyte to its internal standard.
[b]Percent Residual Accuracy.

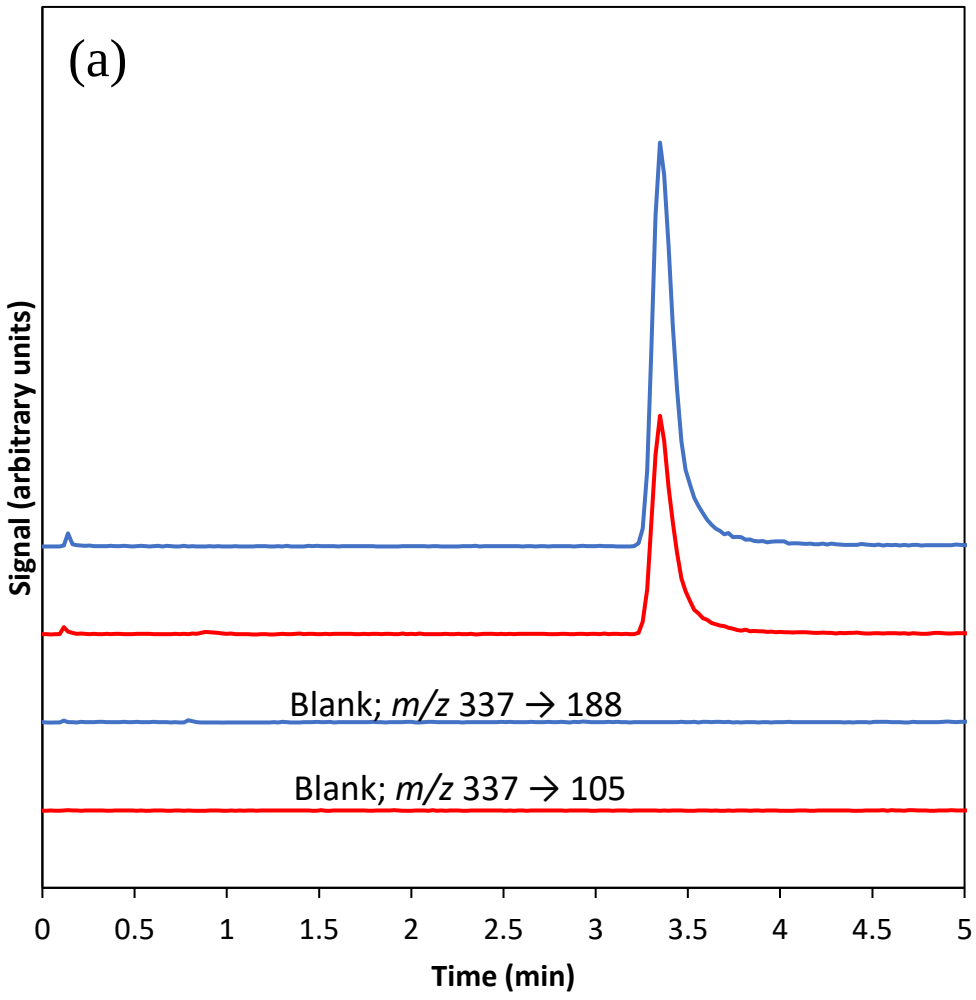
The DI-HPLC-MS/MS method's accuracy and precision were evaluated at three QC concentrations (i.e., 0.5/5.0, 5.0/25, and 25/50 ng/L), and analyzed in quintuplicate over the course of three days (n = 15 for each concentration). Table 5.5 shows the intra- and interassay accuracy and precision of the method. The method generated satisfactory intraassay accuracy for all compounds, ranging between 90 and 115%, with good interassay accuracies between 94 – 110%. Intraassay precision for all compounds was between 1.9 and 11% RSD and interassay precision was between 4.0 and 9.3% RSD. Potential matrix effects were evaluated by comparing the slopes of solvent- and matrix-based calibration curves. A negligible matrix effect for analysis of the opioids in drinking water was found, with $m_{\text{drinking water}}/m_{\text{purified water}}$ of 0.97, 1.01, and 1.02 for fentanyl, hydrocodone, and codeine, respectively. The method recovery for opioids in drinking

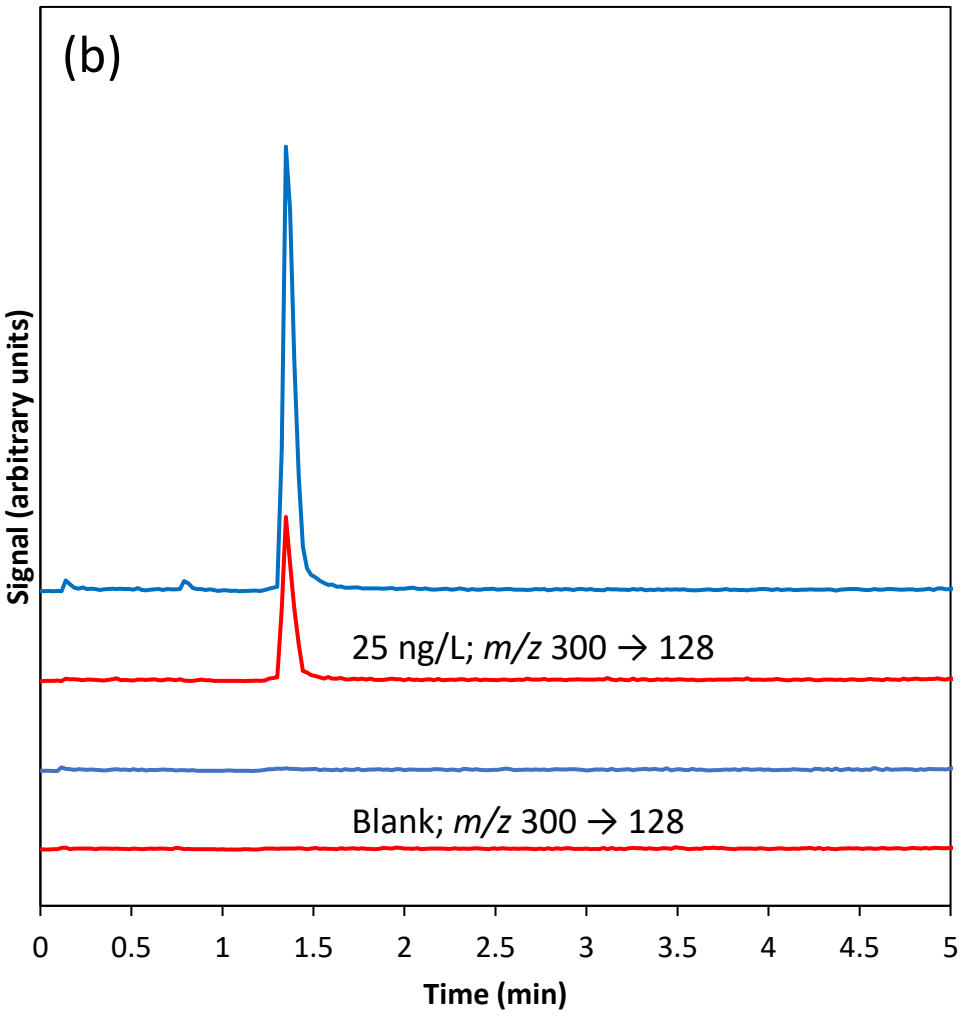
water was quantified at 2.5 and 10 ng/L. Overall, the recoveries were excellent, ranging from 88 – 111% for the low QC and 93 – 113% for the high QC. Specifically, low QC recoveries ranged from 89 – 101% for fentanyl, 100 – 111% for hydrocodone, and 88 – 106% for codeine, respectively, and high QC recoveries ranged for 91 – 99% for fentanyl, 96 – 113% for hydrocodone, and 93 – 113% for codeine. These high recoveries indicate minimal loss of the analytes during filtration.

Table 4.6 Intra- and interassay accuracy and precision of QCs based on 3 days of analysis.

Analyte	Concentration (ng/L)	Intraassay accuracy (%)[1]	Interassay accuracy (%)[2]	Intraassay precision (%RSD)[1]	Interassay precision (%RSD)[2]
Fentanyl	0.5	90 – 101	94	1.9 – 4.0	6.4
	5.0	101 – 109	105	2.3 – 3.9	4.0
	25	105 – 115	110	1.9 – 4.0	4.8
Hydrocodone	5.0	101 – 105	103	6.4 – 7.9	6.9
	25	99 – 106	102	4.5 – 7.2	6.0
	50	100 – 103	102	6.4 – 8.1	7.1
Codeine	5.0	97 – 104	100	9.2 – 9.7	9.3
	25	98 – 102	100	9.0 – 11	9.1
	50	99 – 108	103	7.3 – 10	8.2

[a]Mean of the values for 1 day (n = 5 for each day); overall range for three separate days.
[b]Mean of the values over the three-day validation period (n = 15).


(a)
Signal (arbitrary units)
Blank; m/z 337 → 188
Blank; m/z 337 → 105
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Time (min)


(b)
Signal (arbitrary units)
25 ng/L; m/z 300 → 128
Blank; m/z 300 → 128
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Time (min)

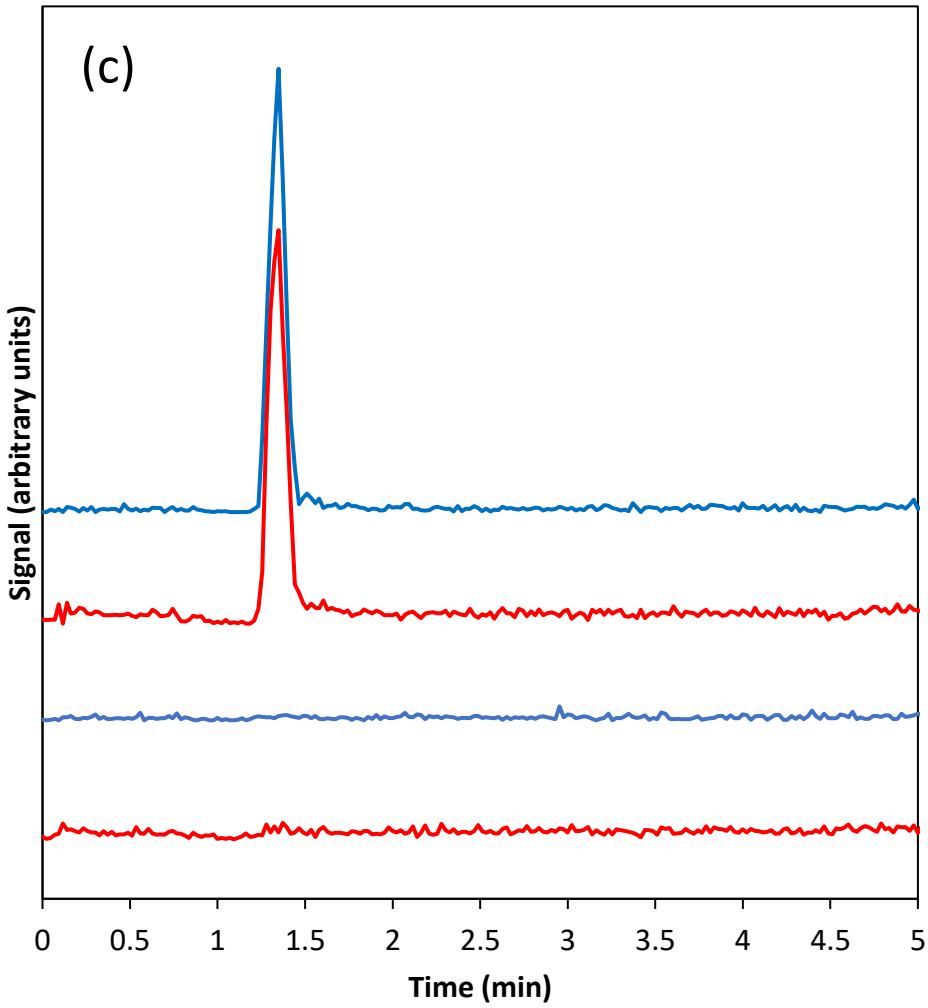


Figure 4.2 Representative chromatograms of opioids in drinking water at 25 ng/L: (a) fentanyl, (b) hydrocodone, and (c) codeine. The quantification (top/blue) and identification (bottom/red) ion are each plotted for the blank and fortified samples.

5.3.2 Prevalence of opioid contamination in U.S. drinking water sources

To determine the prevalence and level of opioid contamination in U.S. drinking water sources and in order to evaluate the newly validated method 14 drinking water samples were analyzed from locations across the U.S., including from South Dakota, Minnesota, California, New Jersey, West Virginia, Iowa, and Oklahoma. Table 5.6 lists the sample locations and the concentration of opioids found for the three opioids tested and Figure 5.3 shows representative chromatograms of the opioids detected from drinking water for two representative samples. Overall, the method detected hydrocodone and codeine in all water samples except three (i.e., opioids were detected in 79% of samples analyzed). Fentanyl was not detected in any of the water samples tested likely due to its relative hydrophobicity (log K_{ow} = 3.4), which should result in greater partitioning into sediment and soil associated with water sources, instead of remaining in water. This is in line with other opioid studies (Table 5.6), with the exception of the Rodayan et al. study.[113] Specifically, for the studies in Table 5.1 that detected fentanyl in drinking water, fentanyl was found in 2.0%,[112] 5.6%,[115] and 100%[113]of the drinking water samples tested. While the Rodayan et al. study[113] generated a much higher prevalence of fentanyl (100%) than the others in Table 5.1, only three samples were analyzed, versus 18 and 50 for the Boleda (2011)[112] and Boleda (2009)[115] studies, respectively. The high prevalence of fentanyl in the Rodayan et al. study[113] was likely due to their use of a polar organic chemical integrative sampling (POCIS) device, which allows for concentrating analytes of interest passively over the course of days to months. This would account for the concentration of fentanyl over time, as POCIS has been shown effective at concentrating and sampling pharmacetuticals from water.[118]

The highest concentrations of hydrocodone and codeine detected in drinking water samples were 2.8 and 20 ng/L, respectively. These opioid concentrations are within the range found by others for European water sources, with Boleda et al.[115] detecting codeine at concentrations as high as 76 ng/L. For hydrocodone, only one other study[102] tested drinking water for this compound, but did not detect it in any samples. For the current study, we found hydrocodone in all samples where codeine was found (79%). Both of these compounds are much more prevalent in U.S. drinking water sources than those tested in other studies (Table 5.1). Codeine and hydrocodone are more soluble in water than fentanyl, likely leading to their increased prevalence in drinking water. Additionally, due to the inherent structural and polarity similarities between codeine and hydrocodone, and their ubiquity in the U.S., it's not a surprise that they were detected in the majority of samples tested.

In the instances where there are more than one water sample from the same city (e.g., Iowa City, IA had three samples), these samples did not come from the same building or tap and represent both residential and commercial locations. However, due to the similar results found between samples from the same city (e.g., Brookings, SD was ND for both samples tested and Minneapolis, MN generated similar results for hydrocodone and codeine for its two samples), it's likely that the water sources, or at least the treatment plant capabilities, are shared within the same city.

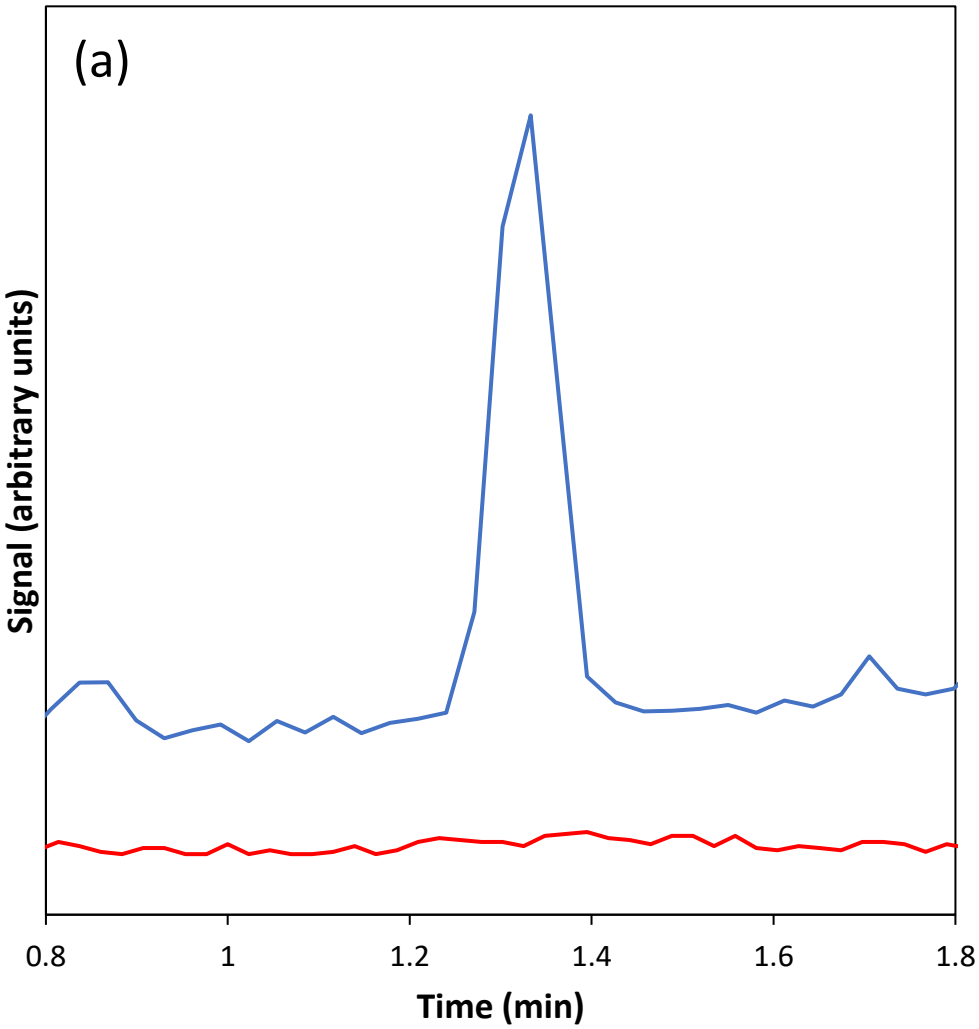
Similar to the other studies listed in Table 5.1, there is a great deal of variability of opioids detected in drinking water sources. The National Institutes of Health (NIH),[119] ranks West Virginia as the state with the highest "opioid involved overdose per 100K persons" (42.4), and specifically Cabell County, WV (where the Huntington water

sample originated), has the highest "opioid prescriptions per 100 person" (92.1) of all the samples analyzed in this work. Although the Huntington, WV sample had detectable values of both hydrocodone and codeine, they weren't the highest concentrations detected in this work. Instead, the sample from Marshall, MN generated the highest values for both analytes. The NIH rates MN, and Lyon county specifically, with "opioid involved overdose per 100K persons" and "opioid prescriptions per 100 person" at 35.5 and 39.0, respectively. The lack of correlation of opioid concentrations in drinking water to local opioid use detected in this work is not surprising, as other studies were also not able to correlate opioid concentrations in drinking water with population, available prescription data or other variables. In fact, this variability could be due in part to uncontrollable variables (e.g., concentrations of opioids in the water sources could fluctuate over time and API removal capabilities of DWTP (Drinking Water Treatment Plants) could vary significantly).

Table 4.7 Field drinking water sample locations and opioid concentrations.

Sample Number	Sample Location	Fentanyl (ng/L)	Hydrocodone (ng/L)	Codeine (ng/L)
1	Brookings, SD #1	--	--	--
2	Brookings, SD #2	--	--	--
3	Volga, SD	--	0.4	1.3
4	Marshall, MN	--	2.8	20
5	Minneapolis, MN #1	--	1.1	5.8
6	Minneapolis, MN #2	--	0.3	2.2
7	Iowa City, IA #1	--	--	--
8	Iowa City IA #2	--	0.5	1.8
9	Iowa City, IA #3	--	0.4	2.1
10	Carson, CA	--	0.4	2.3
11	Oklahoma City, OK	--	1.2	5.5
12	Huntington, WV	--	0.3	1.7
13	Culloden, WV	--	0.5	1.0
14	Fairfield, NJ	--	0.5	2.7

Note: "--" represents not detected.


(a)
Signal (arbitrary units)
0.8
1
1.2
1.4
1.6
1.8
Time (min)

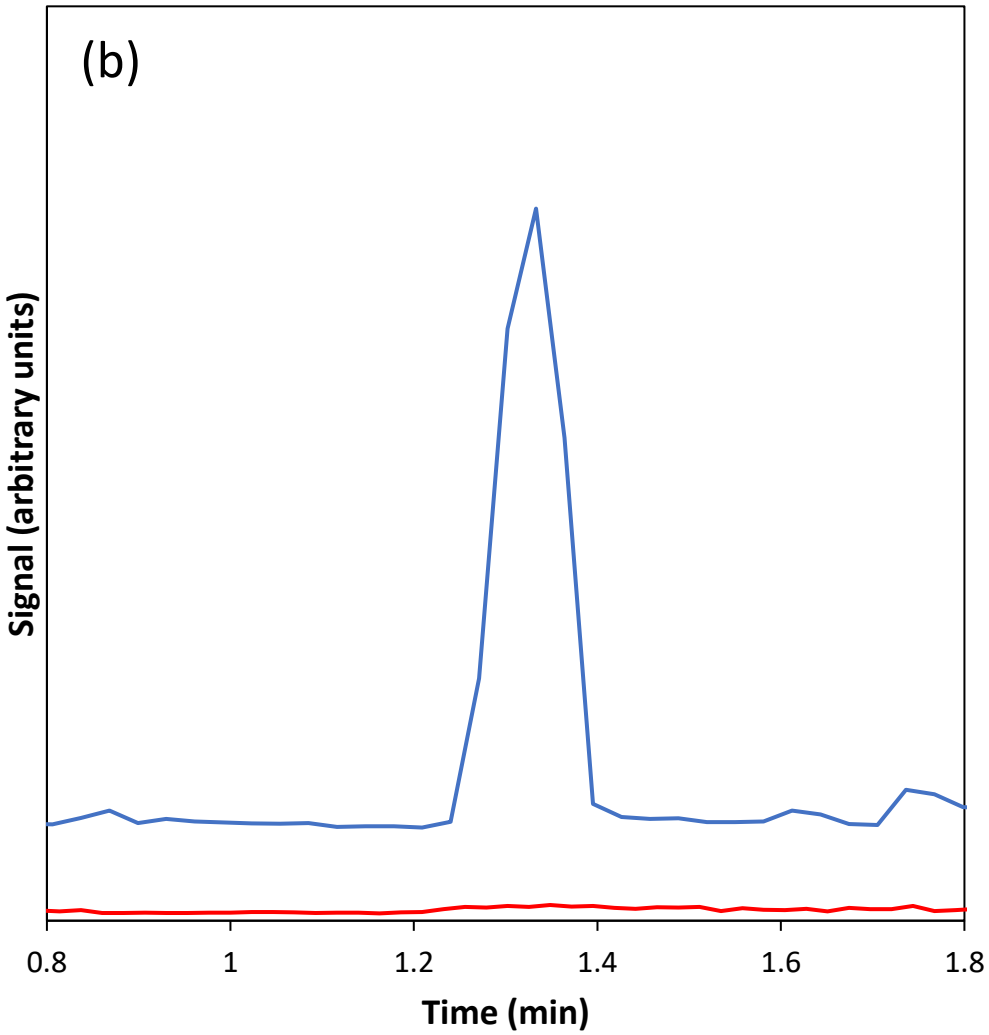


Figure 4.3 Chromatograms for (a) hydrocodone (0.5 ng/L) in Culloden, WV residential drinking water (blue) and a blank drinking water sample (red); (b) codeine (20 ng/L) in Marshall, MN residential tap water (blue) and a blank drinking water sample (red).

5.3.3 Conclusion

As the desire grows to ensure drinking water is safe for consumption, ultratrace methods to detect contaminants, such as opioids, is becoming more important. This simple and highly sensitive DI-HPLC-MS/MS method provided an accurate and precise analysis of fentanyl, hydrocodone, and codeine at ultratrace levels in drinking water. The

method is simple, sensitive, and robust, and detected ultratrace concentrations of hydrocodone and codeine in U.S. drinking water.

The primary goal of this work was to develop, validate, and apply a method to analyze opioids at ultratrace concentrations in U.S. drinking water; however, and possibly more importantly, this showed the prevalence of hydrocodone and codeine contamination in U.S. drinking water sources. This is the first study to analyze U.S. drinking water sources for these opioids[110, 117] with the only other study evaluation of opioids focusing on WWTP effluent and surface water.[120]

The availability of this technique allows simple and reliable detection of these three analytes at sub-ppt concentrations with minimal sample preparation. With this technique, hydrocodone and codeine were consistently detected in drinking water samples at concentrations up to 20 ng/L, in 11 of 14 samples (79%). Fentanyl was not detected in any drinking water sample tested.

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