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ICE CONCENTRATION LINKED WITH EXTRACTIVE STIRRER

(ICECLES)

BY

NUJUD OMAR MASLAMANI

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Chemistry

South Dakota State University

2016

ICE CONCENTRATION LINKED WITH EXTRACTIVE STIRRER

(ICECLES)

This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Chemistry degree and is acceptable for meeting the thesis 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 thesis work is dedicated to my husband, Bander Hamzi, who has been a constant source of support and encouragement during the challenges of graduate school and life. I am truly thankful for having you in my life. This work is also dedicated to my parents, who have always loved, support and encourage and whose good examples have taught me to work hard for the things that I aspire to achieve.

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TABLE OF CONTENTS

ABBREVIATIONS………………………………………………………………….. vii

LIST OF FIGURES…………………………………………………………………ix

LIST OF TABLES…………………………..……………………………………xi

ABSTRACT... xii

CHAPTER 1. INTRODUCTION 1

1.1. SIGNIFICANCE.. 1

1.2. OBJECTIVE 1

1.3. SAFE DRINKING WATER... 2

1.3.1. DRINKING WATER CONTAMINATION AND HEALTH RISK........................ 2

1.3.2. HEALTH RISKS.. 2

1.3.3. TYPES OF DRINKING WATER CONTAMINANTS 3

1.3.3.1. ATRAZINE AND ITS METABOLITES 4

1.3.3.1.1. Uses and Mechanism..4

1.3.3.1.2. Structure and Properties 5

1.3.3.1.3. Toxicity and Mechanism...5

1.3.3.2. METRIBUZIN 7

1.3.3.2.1. Uses and Mechanism..7

1.3.3.2.2. Structure and Properties 8

1.3.3.2.3. Toxicity and Mechanism...8

1.3.3.3. METAMITRON 9

1.3.3.3.1. Uses and Mechanism..9

3.3.3.2. Structure and Properties...10

1.3.3.3.3. Toxicity and Mechanism...11

1.3.4. REGULATING DRINKING WATER...11

1.3.4.1. EPA...11

1.3.4.2. MCLGs and MCLs 11

1.4. CONTAMINANT ANALYSIS OF DRINKING WATER 13

1.4.1. EPA METHODS OF ANALYSIS 13

1.4.2. Gas Chromatography-Mass Spectroscopy...13

1.4.3. SAMPLE PREPARATION TECHNIQUES FOR TRACE ANALYSIS 16

1.4.3.1. Liquid – Liquid Extraction (LLE)...17

1.4.3.2. Solid–Phase Extraction (SPE) 17

1.4.3.3. Solid Phase Microextraction (SPME)...18

1.4.3.4. Stir Bar Sorptive Extraction (SBSE)...18

1.4.3.5. Freeze Concentration (FC)...20

1.5. RESEARCH GOAL.. 21

CHAPTER 2: ICE CONCENTRATION LINKED WITH EXTRACTIVE STIRRER (ICECLES) 22

2.1 ABSTRACT.. 22

2.2. INTRODUCTION 23

2.3. MATERIAL AND METHODS 25
2.3.1. Materials 25
2.3.2. ICECLES Apparatus 26
2.3.3. ICECLES Sample Preparation 27
2.3.4. Gas Chromatography – Mass Spectroscopy 28
2.3.5. Optimization of ICECLES Experimental Parameters 29
2.3.6. Calibration, Limits of Quantification, Signal Enhancement and Limit of Detection .. 30
2.4. RESULTS AND DISCUSSION 31
2.4.1 Sample Preparation with ICECLES 31
2.4.2. Effect of Stir Speed and Freeze Rate 34
2.4.3. Effect of Initial Analyte Concentration 37
2.4.4. Linear range, accuracy and precision 37
2.4.5. Comparison of ICECLES and SBSE 39
2.5. CONCLUSION AND FUTURE WORK 42
CHAPTER 3. DETERMINATION OF TRIAZINE PESTICIDES FROM AQUEOUS SAMPLE USING ICE CONCENTRATION LINKED WITH EXTRACTIVE STIRRER (ICECLES) 43
3.1. ABSTRACT 43
3.2. INTRODUCTION 43
3.2. EXPERIMENTAL 45
3.2.1. Materials and Methods 45
3.2.3. Lower Limit of Quantification, Signal Enhancement and Limit of Detection 46
3.2.4. Instrumentation 47
3.3. RESULTS AND DISCUSSION 50
3.3.1. Comparison ICECLES and SBSE 50
3.3.3. Analysis of pesticides in Source and Treated Drinking Real Water 53
3.4. CONCLUSION 55
CHAPTER 4: CONCLUSION AND FUTURE WORK 57
4.1. CONCLUSION 57
4.2. FUTURE WORK 58

ABBREVIATIONS

ATR: Atrazine

DIA: Deethylatrazine

DEA: Deisopropylatrazine

DMTS: Dimethyl trisulfide

EPA: Environment Protection Agency

FC: Freeze Concentration

ICECLES: ICE Concentration Linked with Extractive Stirrer

SBSE: Stir Bar Sorpitive Extraction

LLE: Liquid-Liquid Extraction

SPE: Solid Phase Extraction

SPME: Solid Phase Microextraction

MTB: Metribuzin

MTT: Metamitron

K_{ow}: Octanol Water Partition Coefficient

SE: Signal Enhancement

LOD: Limit of Detection

LLOQ: Limit of Quantification

f_{LOD}: Fraction of Limit of Detection

RSD: Relative Standard Deviation

Ppb: part per billion

Ppt: part per trillion

QC: Quality Control

R^2: Correlation Coefficient

MCLGs: maximum contaminant level goal

MCLs: maximum contaminant levels

PDMS: Polydimethylsiloxane

DBP: disinfection by-products

TD-GCMS: Thermal Desorption-Gas Chromatography Mass Spectroscopy

CIS: Cooled Injection System

TDU: Thermal Desorption Unit

EI: Electron Ionization

CI: Chemical Ionization

m/z: Mass-to-charge ratio

LIST OF FIGURES

Figure 1.1. A Risk estimation process………...3

Figure 1.2. The chemical structure of metribuzin..8

Figure 1.3. The chemical structure of metamitron...10

Figure 1.4. Schematic for GCMS..15

Figure 1.5. Thermal Desorption Unit (TDU)…...16

Figure 1.6. Stir Bar Sorpitive Extraction schematic...20

Figure 2.1. Schematic of ICECLES apparatus used for the current study............................27

Figure 2.2. ICECLES technique performed on a solution of methyl violet before (A and a), during (B and b), and after (C and c) ICECLES. The intensity of the blue color in the schematic (A-C) indicates the location of the analyte at each stage of ICECLES. In images b and c, the methyl violet is being clearly concentrated in the aqueous solution, with almost clear ice freezing in the bottom portion of the vial (indicating purity of the ice). The location of the extractive stir bar in each image is indicated by a white arrow...34

Figure 2.3. Effect of different coolant temperatures (i.e. freeze rates) on ICECLES for aqueous solution of 100 nM benzaldehyde. SBSE was evaluated using the same sample preparation time as ICECLES...36

Figure 2.4. The calibration behavior of benzaldehyde (40-5000 nM) prepared via ICECLES. The calibration curve is clearly linear with a 1/x weighted fit producing an R^2 of > 0.999...38

Figure 2.5. The performance of ICECLES relative to SBSE for compounds with log K_{ow}s ranging from 0.6 – 2.9. The behavior of signal enhancement (SE) and f_{LOD} are similar, with ICECLES performing better relative to SBSE as the K_{ow} decreases...41

Figure 3.1. Wide range of log k_{ow} (0.83-2.4) for triazine pesticides were tested using ICECLES versus SBSE. The behavior of signal enhancement (SE) and f_{LOD} are relatively similar, with ICECLES performing better relative to SBSE as the K_{ow} decreases...52

Figure 3.2. Pesticide chromatograms for ICECLES and SBSE. Chromatographic peaks are labeled as ICECLES 1) deethylatrazine and deisopropylatrazine, 2) atrazine, 3) metribuzin and 4) metamitron..53

Figure 3.4. Chromatograms for ICECLES and SBSE for application of spiked drinking water before (A) and after (B) treatment with blank for each water for ICECLES. Chromatographic peaks are labeled 1) deethyl-atrazine and deisopropyl-atrazine, 2) atrazine, 3) metribuzin and 4) metamitron..55

LIST OF TABLES

Table 1.1. Chemical properties and structure of Atrazine and its Metabolites …………..5

Table 1.2. Show an example of MCLG and MCL of some contaminants in drinking water…………...13

Table 2.1. Important GC-MS parameters for the five compounds used in this study to evaluate by ICECLES sample preparation……………………………………..….29

Table 2.2. The accuracy and precision of benzaldehyde QCs prepared via ICECLES……………………………………………………………………..…39

Table 2.3. Comparison of SBSE and ICECLES for a range of K_{ow}s………..………………..…………………………………………………..…41

Table 3.1. Important GC-MS parameters for the five compounds used in this study to evaluate by ICECLES sample preparation………......…………………………………......49

Table 3.2. Comparison of SBSE and ICECLES for a range of K_{OW}s pesticides…..……51

ABSTRACT

ICE CONCENTRATION LINKED WITH EXTRACTIVE STIRRER
(ICECLES)

NUJUD MASLAMANI

2016

Clean water is very important for human health. Therefore, drinking water contamination is a significant research concern, especially with the increasing global population. Some contaminants, such as pesticides, can cause health effects even at low levels. Therefore, trace and ultratrace analysis of contaminants in drinking water is essential. Ultratrace analysis typically requires highly effective sample preparation methods and/or highly sensitive instruments. There are currently advanced sample preparations techniques available to analytical chemists that may allow trace analysis of certain analytes. These include liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase micro extraction (SPME), and stir bar sorpitive extraction (SBSE). Even though these techniques have advantages, some analytes are still extremely difficult to analyse at the trace and ultratrace concentrations necessary to ensure safe drinking water. Therefore, a novel sample preparation method was developed as a combination of SBSE and FC to allow trace analysis of drinking water samples. The technique, ICE Concentration Linked with Extractive Stirrer (ICECLES), illustrated great advantages for ultra-trace analysis of multiple analytes. Parameters affecting the performance of ICECLES were evaluated using benzaldehyde, such as the initial concentration, stir speed and freeze rate. Extraction at low speeds resulted in higher extraction efficiency.

However, the freeze rate and initial concentrations had a minor effect on ICECLES extraction efficiency. ICECLES produced linear range of benzaldehyde from 40-5000 nM, with $R^2 > 0.999$, the accuracy was 100 ± 15%, and the precision was ≤ 16% RSD for the QCs. ICECLES provided greater extraction efficiency, signal enhancement (SE) and lower limits of detection (LOD) compared to SBSE method for each analyte tested. ICECLES was evaluated in five different analytes (2-butanol, benzaldehyde, benzyl Alcohol, dimethyl trisulfide and bromobenzene; with log K_{ow} of 0.61, 1.1, 1.48, 1.87 and 2.9, respectively). ICECLES produced the highest SE for 2-butanol, 474 times than SBSE, and the SE generally correlated with decreasing log K_{ow}. ICECLES was shown to be an excellent sample preparation method for analyzing triazine pesticides from aqueous samples. ICECLES achieved greater SE and extraction efficiency for all pesticides, especially for lower log K_{ow} compounds, with trend: metamitron > deethyl-atrazine > deisopropyl-atrazine > metribuzin > atrazine inversely following the log K_{ow}.

Chapter 1. Introduction

1.1. Significance

Over the last few decades, there has been an extraordinary increasing the demand on analytical chemistry techniques, mainly in terms of selectively and sensitivity. Most analysis techniques, which have the ability to meat these demands, such as chromatography, spectroscopy, and mass spectrometry, require pretreatment of analytical samples prior to analysis in order to obtain the required specificity and selectivity especially for ultra-trace detection. Most modern sample preparation techniques, both enrich the analyte and remove interferences in order to transform a sample to a suitable form for analysis. Although current specific sample preparation techniques can analyse many compounds at the required limits, there are many compounds that can not be analyzed without arduous sample preparation (e.g., concentrating very large sample). Therefore, there is a need to develop highly sensitive, environmentally friendly, and easy sample preparation techniques to detect analytes at trace and ultratrace levels, considered as part per billion (ppb) and part per trillion (ppt), respectively.

1.2. Objective

The overall objective of this work was to combine SBSE and FC into ICE Concentration Linked with Extractive Stirrer (ICECLES) as an effective and easy analytical sample preparation technique and demonstrate its application for drinking water analysis. Chapter 2 is the first report of ICECLES and shows its excellent performance as a sample preparation technique compared to SBSE, providing excellent extraction efficiency and signal enhancements (SEs). The use of ICECLES to detect

pesticides in drinking water illustrated lower detection limits and higher signal enhancements verses SBSE and is reported in Chapter 3.

1.3. Safe Drinking Water

1.3.1. Drinking Water Contamination and Health Risk

One of the world's most pressing public health needs is access to safe drinking water. In 2008, an approximately 13% of the world is population lacked access to clean water sources. In the USA, the average person uses approximately 90 gallons of water each day, and more than 107,000 gallons each year. According to the Safe Drinking Water Act, the incidence of cancer, especially breast and prostate cancer, has been increased over the past 30 years, likely due to pollutants found in drinking water [1]. The US Environment Protection Agency (EPA), reported that more than three million of people were exposed to arsenic and other harmful compounds at very low doses through drinking water consumption [1]. Each exposure to toxic chemicals through drinking water carries a corresponding health risk, generally associated with long-term exposure.

1.3.2. Health Risks

According to epidemiologic evidence, there is a correlation between contaminants in drinking water and cancer [2]. The population risk estimate is an assessment of the total estimated excess number of cancer incidents in the US from a given contamination [3]. For example, arsenic in drinking water causes liver, bladder and kidney cancer according to epidemiologic studies, which estimated that 3000 of cancer per year in US [4] This estimate includes the level of daily intake (oral, respiratory, and dermal) resulting from the presence of a contaminant in water as shown in Figure 1. Expert estimates that approximately 10,700 bladder and rectal cancers each year may be

associated with contaminants chemical from drinking water in US [5]. The estimation of individual risk rates is based on bioassay data that is derived from animal experiments.

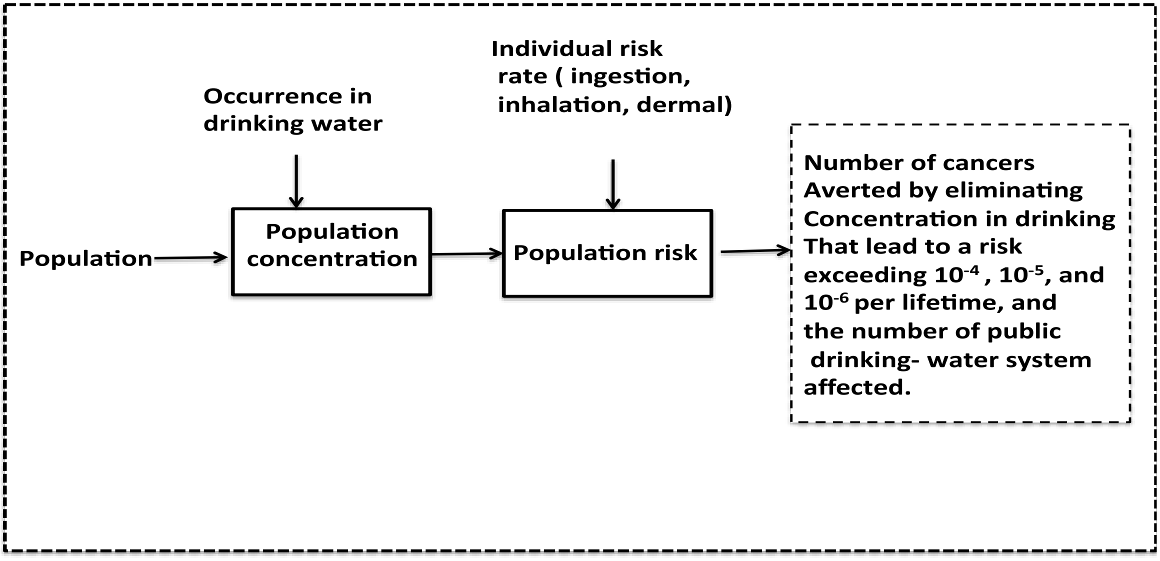


Figure 1.1. Risk estimation process [6]

1.3.3. Types of Drinking Water Contaminants

Many varied contaminants, which may lead to adverse health effects, are of concern for drinking water. Drinking water may contain individual contaminants, or mixtures unique to the medium, such as disinfection by-products (DBP). Some DBP contaminants are unregulated and more toxic than their chlorinated and carbonaceous DBP and may exist in complex mixtures, such as Idonated or nitrogenated DBPs. Moreover, DBP contaminations may occur in water supplies at very low concentrations [7]. Some of the main classes of toxic compounds of concern to drinking water are pesticides and herbicides. There are three well-known classes of pesticides in the US: triazines, organophosphates and carbamates [8]. These are widely used in agriculture and

used as industrial chemicals used in modern society. Therefore, these can contaminate surface water, ground water and drinking water. The pesticides and herbicides of interest to the current study are described below.

1.3.3.1. Atrazine and its Metabolites

1.3.3.1.1. Uses and Mechanism

Atrazine and its chloro-*s*-triazine metabolites, deethylatrazine (DEA) and deisopropylatrazine (DIA), are herbicides widely used globally. It has been used as a component of weed control programs since it was first registered in 1958. In the US, atrazine is used for broad leaf and grassy weed control for crops such as corn, sorghum, sugar cane, pineapple, Christmas trees and conifer restoration. Atrazine is the most popular herbicide in the US and it is applied to around 69% of corn [9]. The estimated use of atrazine in 1993 was 32,000-34,000 tons of active ingredient in North America alone [10]. Atrazine is a component present in many different pesticide mixtures and it is rarely used alone. For example, there are approximately 100 different pesticide products containing atrazine registered with the Minnesota Department of Agriculture. The largest atrazine manufacturer is Novartis and has more than one trade name, including Marksman, Coyote, Atrazina, Atrazol and Vectal. Atrazine is available as dry and liquid flowable, granular, and wettable powder formulations [11].

Atrazine is a mobile herbicide that is absorbed by plants mainly through the roots, but also through the foliage. Once it is absorbed, it is translocated upward and accumulates in the growing tips and the new leaves of the plant. It acts as a photosynthesis inhibiter in plants by preventing electron transfer between photosystem I and II.

1.3.3.1.2. Structure and Properties

Atrazine is part of the s-triazine chemical family. DEA and DIA are biotic degradation products of the parent compound atrazine. Both are persistent and mobile in water; however, DEA is more stable. DEA and DIA are structurally and toxicologically similar to atrazine, as shown in Table 1. Atrazine and its metabolites are not very water-soluble, and are stable under normal temperatures and pressures.

Table 1.1. Chemical properties and structure of Atrazine and its Metabolites [12].

	Atrazine	Deethylatrazine	Deisopropylatrazine
Structure			
Chemical Name	1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine	2-amino-4-chloro-6-(isopropylamino)-s-triazin	6-Chloro-N2-ethyl-1, triazine-2,4-diamine
Formula	$C_8H_{14}ClN_5$	$C_6 H_{10}ClN_5$	$C_5H_8ClN_5$
Molar mass (g/mol)	215.68	187.63	173.63
Water Solubilit (mg/L)	33	3200	670
log K_{ow}	2.60	1.51	1.15

1.3.3.1.3. Toxicity and Mechanism

Exposure to atrazine and its metabolites can occur through farming manufacturing, or consuming contaminated drinking water. In 1990, the EPA classified atrazine as a Restricted Use Pesticide (RUP), and classifies Atrazine as toxicity class III

(moderate toxicity). DEA and DIA have similar effects and modes of action as atrazine. Therefore, most studies have focused directly on atrazine [13]. However, there are a few studies, which have evaluated DEA and DIA. According to one study on these pesticides, acute and chronic toxicity was ranked as atrazine > DEA > DIA, with atrazine only slightly more toxic than its metabolites. The EPA has classified atrazine as an endocrine disruption or neurotoxin carcinogen. Epidemiologic studies have also shown that there is a correlation between atrazine exposure and cancer. Based on case–control studies, atrazine users have shown weak associations with non-Hodgkin lymphoma [14, 15] and increased risk of ovarian and prostate cancers [16]. From 1993-2000, the incidence of cancer among pesticide applicators with exposure to atrazine was evaluated as part of cohort study of approved pesticide applicators in Iowa and North Carolina in the Agricultural Health Study (AHS). No associations between atrazine and leukemia, Hodgkin lymphoma, multiple myeloma [17] and colon cancer [18, 19] were detected. However, there was limited evidence for an association of atrazine use and cancer of the thyroid and ovaries. Similar to humans, atrazine is only slightly toxic to birds. The LD50 in mallard ducks is greater than 2,000 mg/kg.

Once skin exposure to atrazine, it is absorbed and distributed to many different parts of body. Atrazine is mainly metabolized to DEA and DIA. Atrazine remains in the body for few hours, While most of its metabolites, including DEA and DIA, leave the body within 24-48 hours through urine and feces [20]. However, in other studies, atrazine not build up or remain in the body and it quickly eliminated in urine.

1.3.3.2. Metribuzin

1.3.3.2.1. Uses and Mechanism

Metribuzin is a selective herbicide that most widely used in the United States, and in other parts of the world, to control growing and newly developing grasses and broad leaf weeds in soybeans, field crops, and potatoes. Metribuzin is very effective against annual grasses and numerous broadleaf weeds, including cocklebur, velvetleaf, jimson weed and coffee weed. It is applied either during preemergence or early postemergence of weeds. From 1990–1994, the average annual use was estimated at 2.8 million pounds per year of active ingredient, treating approximately 8.5 million acres of soybeans [21-23].

Metribuzin was discovered by Bayer Ag in Germany, and field-testing and development of this herbicide in the United States and Canada was conducted by Mobay Chemical Corporation and DuPont. Metribuzin is sold as a wettable powder containing 50 or 75% metribuzin, under trade names Sencor (trademark of Mobay Chemical Corp) or Lexone (trademark of DuPont) [24]. Metribuzin is absorbed into soils high in organic content and is released from agricultural areas into surface and ground waters during runoff events. It is mainly absorbed by the roots and sometimes by the leaves of treated plants. Metribuzin is then translocated by the xylem and quickly absorbed into mesophyll cells and transported through the cuticle into the cell and chloroplast where it inhibits photosynthesis.

Metribuzin disrupts photosystem II [24-26], binding to the chloroplast D1 thylakoid (membrane) protein, Q_B, and preventing it from accepting and transferring

electrons to the plastoquinone. Finally, Metribuzin binds to the Q_B protein through to one of five binding niches [27, 28].

1.3.3.2.2. Structure and Properties

Metribuzin is a heterocyclic asymmetrical triazine herbicide with systematic name of 4-amino-6-*tert*-butyl-3-(methythio)-1,2,4-triazin-5-one [29]. The structure of metribuzin is shown in Figure 1.2. Metribuzin has two carbon atoms and two nitrogen atoms adjacent to each other. Typically, triazine compounds have symmetrical structures with alternating carbon and nitrogen atoms in the central ring. Its molecular formula is $C_8H_{14}NOS$ and its molecular weight is 214.3 g/mol. Metribuin is a white crystalline solid and is soluble in water up to 1,200 ppm (1.2 g/L). It has a log K_{ow} of 1.70 and a sulfurous odor.

Figure 1.2. The chemical structure of metribuzin.

1.3.3.2.3. Toxicity and Mechanism

The dermal and inhalation toxicity of metribuzin are low. Studies of oral and inhalation exposure have shown that metribuzin has a low toxicity to fish and avian species [21, 30]. The avian oral LD_{50} value ranges from 500 to 1000 mg/kg according to

several studies on bird species, such as bobwhite quail, mallard ducks, canaries and house sparrows [24]. In practice, it is considered non-toxic by the dermal route of exposure. For instance, metribuzin taped to the abraded skin of rats at 20 g/kg for 24 hours did not result in any acute toxic symptoms. Also, the studies reported that the formulated metribuzin did not cause eye or skin irritation or sensitize the skin. It is considered slightly toxic or "moderately hazardous" by the World Health Organization and is listed in Toxicity Category III [21, 30]. The Environmental Protection Agency (EPA) lists metribuzin as a toxicity class IV compound (i.e., lowest toxic category). The EPA has not classified metribuzin as a human carcinogen because of insufficient carcinogenicity data from animal bioassays and the lack of carcinogenicity data in humans [21]. For example, when comparing two groups, (1) 8,504 users of metribuzin with 554 incidents of cancer (6.5%), and (2) 14,568 pesticide applicators who never used metribuzin with 1,118 cases of cancer (7.7%), there was little potential association between metribuzin use and cancer [21]. Although the toxicity is low in most studies, one long-term study of metribuzin toxicity showed toxic effects. After two years of exposure to metribuzin at a rate of 55.7 mg/kg per day, dogs demonstrated a high mortality, decrease body weight and pathologic changes in the liver and kidney [21].

Generally, after metribuzin is absorbed, it is distributed in the body and then excreted in the urine and feces. According to current research, the breakdown of metribuzin in animals and human is not completely understood.

1.3.3.3. Metamitron

1.3.3.3.1. Uses and Mechanism

Metamitron is a selective herbicide used in European countries, but not currently

in the United States. Because of its limited use, toxicity and other information on metamitron is scarce. Metamitron was first developed by Bayer in 1975. It is applied as a spray. It use for eliminating undesirable plants and controlling grasses and broad leaf weeds in sugar bees, fodder beets and certain strawberry varieties. It can be used either pre-emergence or post-emergence. Metamitron is absorbed through the leaf surface and the roots and then translocated to the leaves of the target plants. Metamitron disrupts photosystem II in the plant leaf, leading to inhibited electron transfer.

3.3.3.2. Structure and Properties

Metamitron is an asymmetrical triazine compound. It has chemical name of 4-amine-3-dihydro-3-methyl-6-phenyl-1,2,4-triazine-5-one and the structure of metamitron is shown in the Figure 3. It is a colorless, odorless crystal with formula of $C_{10}H_{10}N_{40}$. It has molecular weight of 202.22 g/mol and log K_{ow} of 0.83. Metamitron is highly soluble in water, leading to high mobility in environment.

Figure 1.3. The chemical structure of metamitron

1.3.3.3.3. Toxicity and Mechanism

Metamitron is slightly toxic and harmful by inhalation and ingestion with reported oral LD_{50} values of 3343 mg kg^{-1} in rats [31], although long-term exposure of metamitron may cause lung irritation and damage [32]. The WHO and EPA classified metamitron as a category II (i.e., slightly hazardous) and category III (i.e., slightly toxic) pesticide, respectively [31].

1.3.4. Regulating Drinking Water

1.3.4.1. EPA

The EPA regulates contamination in drinking water by setting concentration limits as provided by Safe Drinking Water Act (SDWA). The safety of drinking water is ensured by partnership between EPA, the states, tribes, public water systems and their operators. The EPA requires analysis of drinking water collected by the public water system to ensure it is safe to drink. The EPA regulates around 90 contaminants in drinking water. The EPA follows three criteria when establish primary drinking water regulations: 1) contaminants may have adverse effect on health, 2) contaminants are detectable in public water, and 3) contaminants occur in drinking water. The second criteria is very important for analysis since the EPA regulates contaminants, in part, based on the availability of an analytical methods for the contaminant at the level of concern.

1.3.4.2. MCLGs and MCLs

In 1974, the EPA set limits or standards of concentration for certain contaminants in drinking water, which are allowable in public drinking water supplies under SDWA authority. The EPA split these standards into two categories: primary and secondary

standards. Primary standards are set as a level of contaminations that are estimated to cause a health risk or death when present in drinking water. Primary standards are split into inorganic chemicals, organic chemicals, and microorganisms. Secondary standards are set for contaminants that may cause cosmetic and aesthetic effects. The National Primary Standards established a maximum contaminant level (MCL) and a maximum contaminant level goal (MCLG) for each regulated contaminant in drinking water [33]. The MCL is the maximum allowable level of a toxic pollutant in drinking water, while the MCLG is the contaminant maximum exposure level without any health problems related to contamination. Table 1.2. shows some examples of EPA's drinking water standards [33]

Table 1.2. Examples of MCLG and MCLS of some drinking water contaminants

Contaminant	MCLG (mg/L)	MCL (mg/L)	Potential Health Effects	Sources of Contaminant in Drinking Water
Alachlor	Zero	0.002	Eye, liver, kidney or spleen problems; anemia; increased risk of cancer	Runoff from herbicide used on row crops
Atrazine	0.003	0.003	Cardiovascular system or reproductive problems	Runoff from herbicide used on row crops
Benzene	Zero	0.005	Anemia; decrease in blood platelets; increased risk of cancer	Discharge from factories; leaching from gas storage tanks and landfills
Arsenic	0	0.010	Skin damage or problems with circulatory systems and increased risk of getting cancer	Erosion of natural deposits; runoff from orchards, runoff from glass & electronics production wastes

1.4. Contaminant analysis of Drinking Water

1.4.1. EPA Methods of Analysis

The EPA strictly enforces regulations for trace concentrations of many environmental contaminants in water and has some specific sample preparation techniques for trace and ultratrace compounds. Most of the methods use the analysis "gold standard methods" such as gas chromatography-mass spectroscopy and liquid chromatography-mass spectroscopy [34]. For example, the first method (200.8) developed by EPA was titled, "Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma Mass Spectrometry" [35].

1.4.2. Gas Chromatography-Mass Spectroscopy

Gas chromatography-mass spectrometry (GC-MS) is one of the most used

analytical instruments for trace analysis. GC-MS is widely used to separate, identify and quantify chemicals compounds in complex mixtures. As shown in Figure 1.3, the sample is first injected into the GC inlet. The analyte is then vaporized and swept onto a column by carrier gas such as helium, nitrogen or hydrogen. Separation of the analyte in the column depends on several factors, such as temperature, stationary phase, carrier gas and flow rate.

Following GC separation, analytes are sent to an MS detector where they are fragmented and converted into ions in the ion source. There are two common ionization techniques for GC: electron ionization (EI) and chemical ionization (CI). In EI, the fragmentation of chemical compound occurs via ionization with a high-energy (70 eV) beam of electrons. The fragmented ions can be used to determine the structure of the analyte. CI can be used as alternative for El, in cases the analyte ions cannot be detected with EI. CI is a softer ionization and requires reagent gases such as methane. CI produces less energetic collisions and less fragmentation as compare to EI. However, more structural information is obtained from EI than CI.

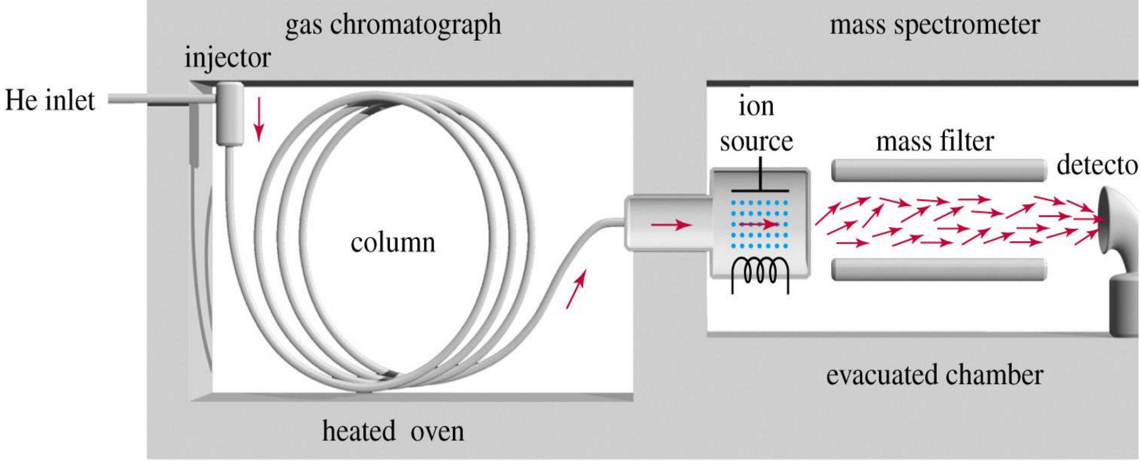


Figure 1.4. Schematic for GCMS [36].

After compounds are fragmented, they enter the mass spectrometer, which is a sensitive and effective detector for GC. There are several mass analyzers, including quadrupole, ion trap, magnetic sector and time of flight. A quadrupole contains four parallel rods, which are responsible for separating ions based on their mass to charge ratio (m/z). A direct current (DC) applied across the rods in combination with an RF potential to acts as filter to allow particular m/z fragments to pass through the detector.

Multiple sample preparation methods can be used to prepare samples for GC analysis including thermal desorption (TD). The thermal desorption system consists of four parts: 1) the thermal desorption unit (TDU) is shown in Figure 1.4., 2) a cooled trap (e.g. a Cooled Injection System (CIS)), 3) transfer lines, 4) some types of valves.

A TDU can be fit on the top of any GC instrument, and it is perfectly suitable for the most types of samples such as liquids, semi-liquids, solids and stir bars can be thermally desorbed. CIS contain deactivate an empty glass liner, was used as cryofocused the

analytes after thermally desorbed in TDU and prior transfer to analytical column. TDU can ether operates in split or splitless mode.

Figure 1.5. Thermal Desorption Unit (TDU) [37].

1.4.3. Sample Preparation Techniques for Trace Analysis

Sample preparation is one of the most important steps in analytical procedures to analyze drinking water. Drinking water contaminants present at trace concentrations require sample preparation techniques with ability to pre-concentrate analytes prior to the analysis, thereby increasing the sensitivity of the overall analysis technique. Moreover, this step involves cleanup procedures to improve the selectivity of the overall analytical method. There are several sample preparation techniques common to the analytical chemistry laboratory that are used for drinking water analysis, such as liquid-liquid

extraction (LLE), solid phase extraction (SPE), solid phase micro extraction (SPME) and stir bar sorpitive extraction (SBSE).

1.4.3.1. Liquid – Liquid Extraction (LLE)

LLE is a sample preparation technique, which has been used in chemical laboratories for centuries. LLE, also known as solvent extraction, uses two immiscible solvents in a container to partition an analyte between the two phases. Partitioning of the analytes between these phases is based on its intermolecular interactions with the solvent. Phase transfer substances can be used to help transport components that cannot partition well into the desired solvent. Because the low cost and simplicity of LLE, it is still used in most EPA methods for analysis of contaminants [38]. LLE is not environmental friendly because of the large volume of organic solvent required. LLE is lengthy, labor-intensive, and may require multi-stage operation [39]. LLE is not suitable and less effective for most polar analytes [38].

1.4.3.2. Solid–Phase Extraction (SPE)

SPE is a separation method where a liquid is passed through a solid sorbent where analytes are portioned or adsorbed, thereby separating them from matrix interferents [38, 40]. SPE is widely used in analytical laboratories because it allows rapid and selective sample preparation. SPE uses differences in sorbent affinity between the analyte and interferents that are present in the liquid phase. This difference in affinity allows the isolation of the desired analyte from the interferents. SPE has four steps: 1) activation of the sorbent by choosing the right solvent to condition the sorbent (e.g., hexane, methanol and/or water), 2) loading the entire sample, including the desired analyte, such that analytes are retained on the sorbent, 3) washing and rinsing the sorbent to remove

impurities, and 4) the desired analyte is eluted from the SPE sorbent and collected for further analysis. SPE offers advantages such as small solvent volumes and it is reproducible and easy [41]. Disadvantage of SPE include long preparation times and multi-step procedures [39].

1.4.3.3. Solid Phase Microextraction (SPME)

SPME was developed in the early 1990s by Arthur et al. [42, 43] and was the first solventless extraction technique for organic compounds. In SPME sampling, extraction, concentration and transfer occur in one-stage and one device [39, 44] making SPME a rapid, simple and efficient sample preparation procedure. SPME is typically based on an equilibrium partitioning of analytes from the liquid phase into the sorbent phase based on a compound's partition coefficient ($K_{sorbent}$). A thin solid fused silica fiber is coated with a polymeric stationary phase such as polydimethysiloxane (PDMS). In general, a SPME fiber is exposed to the aqueous solution containing an analyte. The analyte partitions into the sorbent phase of the fiber. Once equilibrium is reached, the fiber is removed, and introduced into a hot injection port of a GCMS for analysis. SPME has proven to be very adaptable and has been used for many applications including analysis of volatile environmental analytes. However, the amount of extraction phase is very small. The most wildly used fiber is 100 μm PDMS, which corresponds to an extraction phase volume of approximately 0.5 μL. Therefore, the extraction efficiency for solutes that are partially water soluble is quite low [45].

1.4.3.4. Stir Bar Sorptive Extraction (SBSE)

SBSE was first introduced in 1999 by Baltussen et al. [46] as a solvent free sample preparation method for trace organic compounds. As with SPME, SBSE is based

on partitioning [47]. SBSE is an environmentally friendly alternative to liquid extraction. The sorbent in SBSE is coated on a magnetic stir bar. The most widely used sorptive extraction phase is used polydimethylsiloxane (PDMS), which is well-known as a stationary phase in GC. SBSE is performed by adding the coated stir bar into a vial containing a suitable amount of sample. The sample is stirred for 30 – 240 min. After the extraction is complete, the solutes can be introduced quantitatively into a GCMS using by TD or back-extraction into a liquid solvent. The former process has high sensitivity because the TD and GC can analyze the entire extract. SBSE has the advantage of a large amount of extractive phase, 50-250 times larger than SPME [45]. According to recent studies, the SBSE partition coefficient is correlated with the octanol-water distribution coefficient (K_{ow}). Even though not exactly correct, K_{ow} gives a good sign of whether, and how well, the desired analytes can be extracted with SPME or SBSE. Besides K_{ow}, the efficiency of sorptive extraction depends on the phase ratio (β). The distribution coefficient between PDMS and water ($K_{PDMS/w}$) is the ratio of the concentration of a solute in the PDMS phase (C_{PDMS}) to the concentration of water (C_w) at equilibrium as shown in Equation 1.

$$K_{ow} \simeq K_{PDMS}/_{w} = \frac{C_{PDMS}}{C_w} = \frac{m_{PDMS}}{m_w}\,\frac{V_w}{V_{PDMS}} = \frac{m_{PDMS}}{m_w}\,\beta \tag{1}$$

where $K_{PDMS}/_{w}$ is equal to the ratio of the mass of the solute in the PDMS phase (m_{PDMS}) to the mass of the solute in the aqueous phase (m_w) multiplied by β (where $\beta = V_w/V_{PDMS}$).

According to many applications in environmental, food and biomedical fields that applied SBSE in trace analysis, SBSE has extremely high sensitivity. SBSE is well-suited for multiresidue analysis [48]. However, SBSE is not effective with lower K_{ow} analytes

because the hydrophobic characteristic of the PDMS coating.

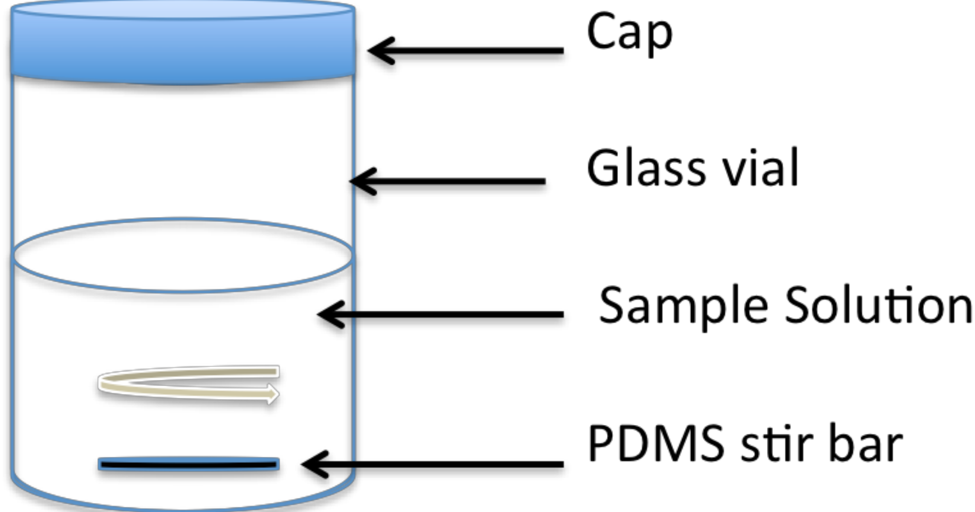


Figure 1.6. Schematic of stir bar sorpitive extraction

1.4.3.5. Freeze Concentration (FC)

FC is a technique that has been used for decades to produce concentrates in the beverage industry, including ice beer and fruit juices. In the last 30 years, more than twenty commercial freeze concentration systems have been developed in the US food product industry [49]. FC is beneficial as it entraps volatile and heat-sensitive components, which are required for aroma and nutrition, in a concentrated solution, whereas they are lost when using heat [49]. The principle of FC is to separate the solute from solution under freezing conditions. Water is partially frozen to form an ice crystal as the solute is expelled into the liquid phase. Advantages of FC include less energy consumption compared to other methods and it is relatively inexpensive. FC is

considered a suitable method for heat-sensitive components in food products and volatile analytes in wastewater [49].

1.5. Research Goal

The ultratrace analysis of some drinking water contaminants to ensure safe drinking water is currently extremely difficult to achieve, especially for volatile and thermally unstable analytes. Therefore, there is a critical need to develop a sensitive and selective sample preparation technique for ultratrace analysis of contaminants, including volatile and thermally unstable analytes. In this study, SBSE and FC were combined in one technique, ICECLES. ICECLES was evaluated for the analysis of multiple analytes, characterized as a novel sample preparation technique, and used to detect contaminants from drinking water.

Chapter 2: ICE Concentration Linked with Extractive Stirrer (ICECLES)

2.1 Abstract

Trace and ultra-trace analysis can be difficult to achieve, especially for non-polar, volatile, and/or thermally unstable analytes. A novel technique, coined ICE Concentration Linked with Extractive Stirrer (ICECLES), may address this problem. The implementation of ICECLES described here, combines stir bar sorpitive extraction (SBSE) with freeze concentration (FC), to extract analytes from aqueous solution by slowly freezing water to concentrate analytes into a polydimethlsiloxane (PDMS) coated stir bar. Five probe molecules, 2-butanol, benzyl alcohol, benzaldehyde, dimethyl trisulfide and bromobenzene were prepared from aqueous solutions using ICECLES. Thermal desorption gas–chromatography mass–spectrometry was then used to quantify these analytes. Parameters affecting the performance of ICECLES were evaluated, such as the initial concentration, stir speed and freeze rate. Extraction at low speeds resulted in higher extraction efficiency. However, the freeze rate and initial concentrations had a minor effect on ICECLES extraction efficiency. ICECLES produced signal enhancements of up to 474x SBSE, indicating much higher extraction efficiencies than SBSE alone. ICECLES also provided lower LODs and excellent reproducibility. Overall, the ICECLES technique was excellent at preparing aqueous samples for trace analysis and shows promise as a novel analytical sample preparation technology.

2.2. Introduction

Sample preparation is an extremely important step in most analyses, especially when trace or ultra-trace analysis (i.e. defined here as low ppb to ppt and ppb for ultra-trace and trace analysis, respectively) is necessary. Analytical sample preparation serves two important primes functions: preconcetration and isolation of analytes from matrix interferences. Several advanced sample preparation techniques that serve these two functions have been introduced in recent years, such as solid phase extraction [50], solid phase microextraction [51], and stir bar sorpitive extraction (SBSE) [52].

SBSE is a solvent-free sample preparation technique first reported in 1999 by Baltussen et al. [52]. SBSE has since been used in many different applications areas, including environmental analysis [53], food analysis [54], and biomedical analysis [54, 55]. In SBSE, analytes are generally extracted from a solvent into a sorbent phase bound to a stir bar. The amount of solute sorbed into the stir bar is governed by its affinity for the stir bar coating, typically polydimethylsioxane (PDMS), as quantified by the partition coefficient of the solute between the sorbent phase and the aqueous phase. After extraction, the analytes associated with the SBSE stir bar can be either thermally desorbed [53] or back-extracted into a liquid solvent [53, 55] for analysis. Because, PDMS is a hydrophobic phase, only highly nonpolar analytes are efficiently extracted [53]. In some studies, SBSE has produced low sensitivities for environment and biomedical matrices (lower than 1 ng/L and 1 μg/L, respectively [53]). Moreover, SBSE has produced LODs down to around 0.01 ng/L (ppt) [53, 56], for certain highly non-polar compounds (i.e., log k_{ow}s greater than 5).

Another, less well-known, sample preparation technique is called freeze concentration (FC). FC is a process where solutes are concentrated in the liquid fraction of a solvent as it is slowly (and progressively) frozen. The fundamental concept responsible for freeze concentration is freezing point depression, where a solvent volume with lower solute concentration is frozen first because of its higher freezing point. This leaves the remaining solution more concentrated.

FC has been used for over 3 decades [49] in the food processing industry to concentrate fruit juice, for ice beer production and coffee extraction [57-60]. It has also been suggested as a means of industrial wastewater and petroleum waste remediation [60-63], purification of drinking water or groundwater, and the production of fresh water from seawater [64, 65]. A common implementation of FC occurs with a sample vessel lowered into cold bath with an overhead stirrer rapidly mixing the solution in the vessel [66]. An ice crystal forms from the bottom of the vessel and grows toward the top as the vessel is lowered into the cold bath [66].

Freeze concentration has advantages compared to other concentration methods, including lower energy requirements, long operational duration, and its relatively low cost [65]. FC can also be beneficial for certain analytes that may be difficult to concentrate by other means. For example, volatile and/or thermally unstable analytes are more likely to be retained in the remaining solvent as compared to heat treatment [67-70]. However, FC can be time consuming, some implementations of FC require complex equipment [71], and FC is not selective [72].

FC and SBSE appear to be highly complementary, with SBSE providing selectivity and the vigorous stirring necessary for FC, and FC providing more

concentrated solutes as the solvent is frozen, which should increase the extraction efficiency. Moreover, operation at low temperatures may allow analysis of compounds with higher volatility and thermally labile components. Reported for the first time here, FC and SBSE were combined into a technique called ICE Concentration Linked with Extractive Stirrer (ICECLES) with evaluation of ICECLES compared to SBSE for a variety of analytes.

2.3. Material and Methods

2.3.1. Materials

All reagents were of LC-MS grade unless otherwise specified. Benzaldehyde (C_7H_6O), 2-butanol ($C_4H_{10}O$), dimethyl trisulfide (DMTS, $C_2H_6S_3$, ≥ 98%), and bromobenzene (C_6H_5Br) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Benzyl alcohol (C_7H_8O) and methanol (MeOH, 99%) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Purified water was obtained from a water PRO PS polisher (Labconco, Kansas City, KS, USA) at a resistivity of 18.2 MΩ–cm.

Aside from bromobenzene and DMTS, stock solutions (100 mM) were prepared in 2 mL of purified water. Because of the low solubility of bromobenzene and DMTS, they were initially diluted in methanol to 100 mM and 400 mM, respectively. The resulting solutions of bromobenzene and DMTS were stored at room temperature and 4 °C, respectively. Test solutions were prepared by serially diluting the stock solutions with purified water to the desired concentration for individual experiments. Note: If the sample solution contained significant amounts of methanol (≥0.2%), the uniformity of the ice produced during FC was disrupted and the effectiveness of FC decreased. Therefore, wherever possible, methanol was not used in preparing stock solutions and standards.

2.3.2. ICECLES Apparatus

A schematic of the ICECLES sample preparation apparatus used for this study is shown in Figure 1. The ICECLES apparatus contains five main parts: 1) a circulating chiller, 2) a double-walled (or "jacketed") beaker, 3) a magnetic stir plate, 4) a sorptive stir bar, and 5) a glass sample vial (24 mL). The chiller used in this study was an LS-Series Compact Chiller purchased from Cole–Parmer (LS5IM11A110C; Bunker Court Vernon Hills, IL, US). The magnetic stir plate (18 cm x 18 cm) was purchased from Thermo Scientific (SP195025; Hanover park, IL, USA). The double-walled beaker and 24 mL vials (with 23 mm diameter) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Sorptive stir bars for SBSE (Twister™, 10 mm) were coated with a 0.5 mm thick polydimethylsiloxane (PDMS) layer (Gerstel, Linthicum, MD, USA). Note: It was important to ensure the vials used for both SBSE and ICECLES did not have a rounded bottom, because the stir bar would collide with the vial wall in rounded bottom vials and break when stirring at the high speeds necessary for ICECLES.

A double-walled glass beaker was placed in the center of a magnetic stir plate. The circulating chiller was connected with the double-walled beaker by silicone tubing and a cooled mixture of 50:50 ethylene glycol and water (typically -7 °C) was circulated through the beaker. A small amount of methanol (1 mL) was added to the bottom of the double-walled beaker to form a thin layer of solvent, which ensured maximum thermal contact between the bottom of the sample vial and the bottom of the beaker.

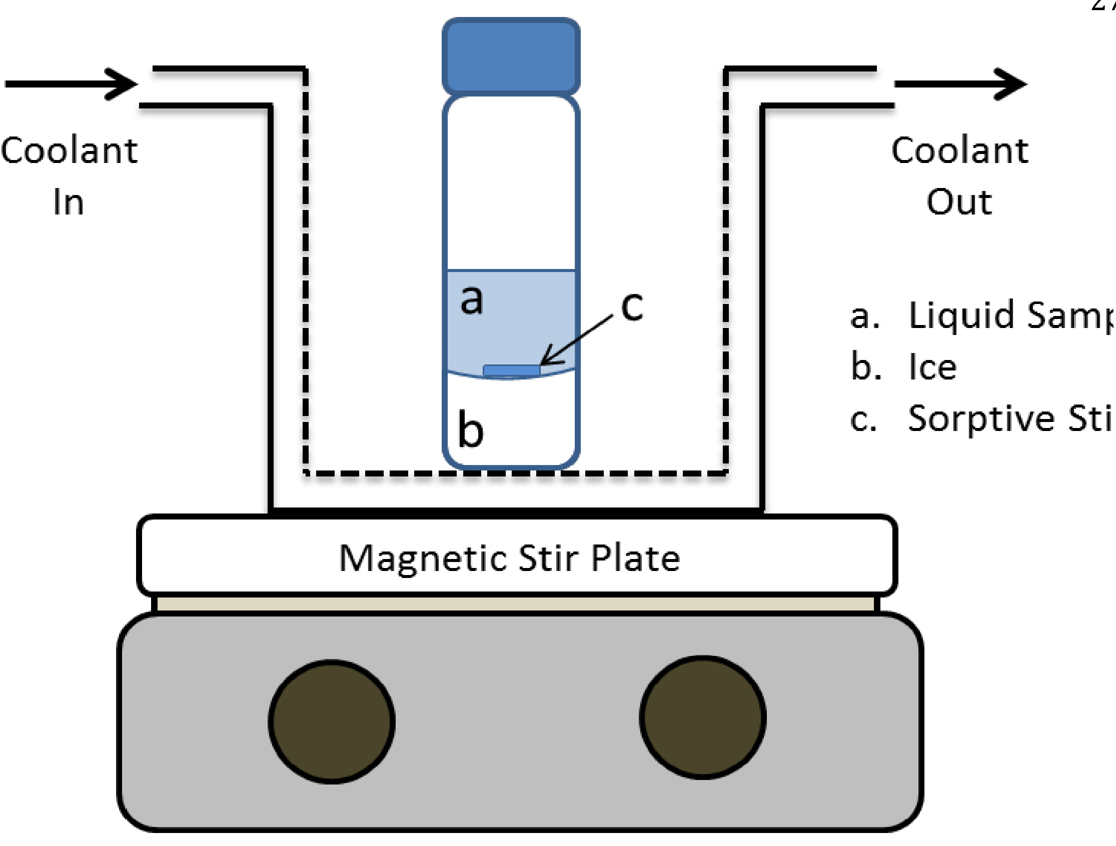


Figure 2.1. Schematic of ICECLES apparatus used for the current study.

2.3.3. ICECLES Sample Preparation

To a glass vial (24 mL), a water sample (10 mL) containing the analyte of interest at the desired concentration and a sorpitive stir bar were added. The vial was capped and placed into the double-walled beaker, coolant was circulated, and the stir plate was set to the desired stir rate. The aqueous sample began freezing progressively from the bottom of the vial steadily upwards until the entire aqueous sample was frozen. The SBSE stir bar remained stirring on top of the ice throughout this process. When optimized, the water sample froze in 3.5 – 4 hours. After extraction, the stir bar was magnetically removed

from the vial with a clean Teflon-coated stir bar. The stir bar was then placed into a thermal desorption (TD) tube and analyzed via TD-GCMS. Analyses were completed in triplicate for each analyte. One a disadvantage of the implementation of ICECLES used for this study is that the sample volume was limited to 10 mL. This because the stir bar moved away from the magnetic field of the stir plate as the ice front moved towards the top of the vial. As the volume increased over 10 mL, the stir plate was no longer able to efficiently spin the stir bar.

2.3.4. Gas Chromatography – Mass Spectroscopy

Prepared stir bars were analyzed using an Agilent Technologies 7890A gas chromatograph and a 5975C inert XL electron ionization (EI)/chemical ionization (CI) mass selective detector (MSD) with Triple-Axis Detector and Multipurpose-Sampler (MPS). Following ICECLES, stir bars were thermally desorbed in splitless mode into a cooled injection system (CIS) liner. Thermal desorption was initiated at 60 ºC, (held for 0.20 min), increased linearly at 720 ºC /min to 250 ºC, and then held constant at that temperature for 1 min. During this process, the analyte was collected in the CIS at 30 ºC. The analyte was transferred from the CIS to the GC column by maintaining the CIS temperature at 30 ºC for 0.20 min and then linearly heating to 250 ºC at a rate of 12 ºC/s. The compounds were separated on a HP-5MS capillary column (30 m x 250 µm x 0.25 µm) with nitrogen as the carrier gas at a flow rate of 1 mL/min and a pressure of 5.565 psi. The temperature gradient for GC separation optimized for each analyte is reported in Table 1, along with the run times for each analyte. For all analytes, the MS source and MS quadruple temperatures were 280 ºC and 150 ºC, respectively. Electron ionization (EI) was used as the ionization source at 70 eV energy. Selected ion monitoring was used

for detection with identification and quantification ions as reported in Table 2.1.

2.3.5. Optimization of ICECLES Experimental Parameters

Optimization of ICECLES for extraction efficiency was accomplished by varying the stir speed and coolant temperature. Benzaldehyde (100 nM) was used as a representative analyte for the optimization. In order to investigate the effect of stir speed on extraction efficiency, multiple stir speeds (1200, 1400 and 1740 rpm) were evaluated. Coolant temperatures were also varied (-3, -5, -7 and -9 ºC) to evaluate the effect of the freeze rate. The effect of initial analyte concentration was evaluated by varying the benzaldehyde concentration between 100 and 3000 nM (10.6 and 282 ppb, respectively).

Table 2.1. Important GC-MS parameters for the five compounds used in this study to evaluate by ICECLES sample preparation.

Compound	Thermal Gradient for GC			Run-Time (min)	Elution-Time (min)	Ion Mass (m/z)	
	Initial	Ramp 1	Ramp 2			Quantification	Identification
2-butanol	30 ºC (2 min)	20 ºC/min to 150 ºC (0 min)	—	5.0	0.6	45	59
Benzyl Alcohol	30 ºC (1min)	90 ºC/min to 180 ºC (1 min)	120 ºC/min to 250 ºC (1 min)	5.2	3.4	91	108
Benzaldehyde	30 ºC (1min)	30 ºC/min to 120 ºC (0 min)	120 ºC/min to 250 ºC (1 min)	6.1	3.8	79	107
Dimethyl trisulfide	30 ºC (1min)	120 ºC/min to 250 ºC (1 min)	—	3.8	2.8	94	126
Bromobenzene	30 ºC (1min)	90 ºC/min to 180 ºC (3 min)	—	5.6	2.7	157	77

2.3.6. Calibration, Limits of Quantification, Signal Enhancement and Limit of Detection

The calibration behavior of benzaldehyde was tested to determine the linear behavior of ICECLES along with the upper limit of quantification (ULOQ). The ULOQ was selected using precision and accuracy inclusion criteria: calibrators with a precision ≥ 15% (measured by relative standard deviation (RSD)), and/or an accuracy of over 100 ± 15% as compared to the nominal concentration calculated from calibration curve were excluded from the linear range. Aqueous calibration standards were prepared in the range of 40-5000 nM from a 100 mM stock solution. Each calibration standard was analyzed in triplicate within a 24-hour period. QCs standards (N=5) were prepared at three different concentrations, 75 nM (low QC standard), 300 nM (medium QC standard) and 1500 (high QC standard). The QC standards were analyzed in quintuplicate during one day using the ICECLES sample preparation process described above. The accuracy of each QC was calculated as a percent difference from the nominal concentration. The precision was calculated as a percent relative standard deviation (% RSD) of the calculated concentration for each QC standard.

The lower limit of quantification for SBSE and ICECLES (benzaldehyde only) was determined by evaluating multiple concentrations of each analyte and selecting the concentration that reproducibly produced a signal to noise ratio of 10, with noise measured as the peak-to-peak noise of the blank over the elution period of the respective analyte. Utilizing the LLOQ for SBSE, ICECLES was performed in triplicate and the signal enhancement (SE) was calculated by dividing the average peak area of ICECLES by the respective average of peak area of SBSE for each analyte. For both SBSE and

ICECLES, the LOD was defined as the lowest analyte concentration that reproducibly produced a signal-to-noise ratio of 3. The limit of detection (LOD) for both ICECLES and SBSE was estimated by the analysis of multiple concentrations of each compound below their LLOQ. The LOD enhancement (f_{LOD}) was calculated by dividing the LOD for SBSE by the LOD for ICECLES under the same conditions, aside from freezing the solvent (i.e., SBSE was completed on the same standard solution as ICECLES, but was performed in a vial directly placed on a stir plate, stirred for the entire ICECLES sample preparation time).

2.4. Results and Discussion

2.4.1 Sample Preparation with ICECLES

ICECLES is a simple and easy sample preparation technique well-suited for trace analysis of water samples. In this study, ICECLES was implemented as the combination of FC and SBSE. For SBSE, a sorpitive stir bar is added to a sample and stirred. Analytes partition into the stir bar, thereby concentrating them in a small volume for later extraction and analysis. Since SBSE is typically performed using PDMS -coated stir bars, only highly hydrophobic compounds are efficiently extracted (i.e., a log $K_{ow} > 3$ allows ≥ 90% extraction of the analyte in the stir bar). For ICECLES, a sorbent-coated stir bar is added to an aqueous sample and stirred, just as with SBSE, but the sample is frozen from the bottom of the vial to the top. Because of freezing point depression, as the solution is frozen, microvolumes of solution with lower solute concentration are frozen first. This concentrates the analyte in the remaining solution, which adds concentration stress to the equilibrium between the analyte in the solution and in the stir bar. This equilibrium shift increases the concentration of the analyte in the stir bar as the solution is frozen.

Somewhat surprisingly, if the freezing is controlled and the stir rate is vigorous, the surface of the ice remains relatively flat and the stir bar remains stirring on top of the ice layer. This allows easy removal of the stir bar from the top of the frozen sample and the ability to freeze the entire sample.

A striking visual example of ICECLES is shown in Figure 2, where a purple dye, methyl violet, was used to simulate an analyte. The capital letters in Figure 2 represent the different stages of ICECLES in a schematic depiction, whereas the corresponding lower-case letters show images of ICECLES sample preparation of an aqueous solution of methyl violet (the position of the stir bar in Figure 2a-c is indicated by the white arrow). The start of ICECLES is shown in Figure 2A and 2a, where a stir bar is added to an aqueous solution with analyte, just as with SBSE. After some time, half the aqueous solution is frozen and the methyl violet is concentrated in the remaining solution and the stir bar (Figure 2B and 2b). Essentially pure water forms the frozen solid as indicated by the nearly clear ice in Figure 2b. At the end of ICECLES sample preparation (Figure 2C and 2c), the methyl violet is concentrated in the stir bar and the last layer of frozen water. The stir bar remains on the top of the ice for easy removal and subsequent analysis.

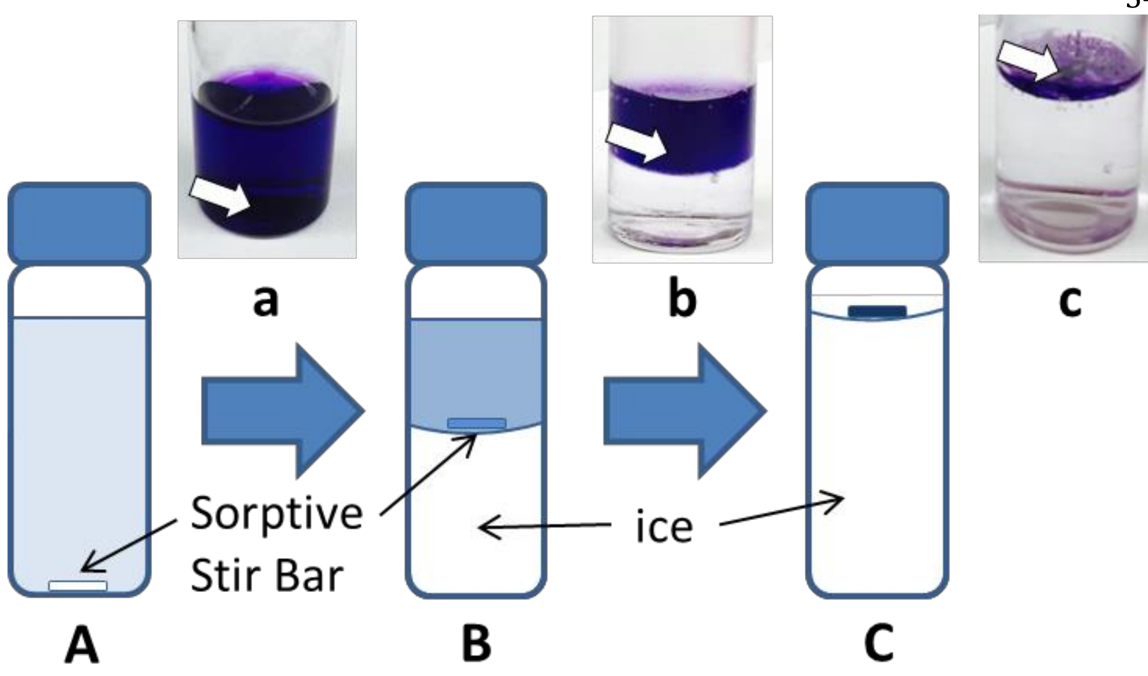


Figure 2.2. ICECLES technique performed on a solution of methyl violet before (A and a), during (B and b), and after (C and c) ICECLES. The intensity of the blue color in the schematic (A-C) indicates the location of the analyte at each stage of ICECLES. In images b and c, the methyl violet is being clearly concentrated in the aqueous solution, with almost clear ice freezing in the bottom portion of the vial (indicating purity of the ice). The location of the extractive stir bar in each image is indicated by a white arrow.

2.4.2. Effect of Stir Speed and Freeze Rate

The experimental parameters important to the current implementation of ICECLES were stir speed and freeze rate. According to most studies of FC, higher stirring velocities result in more efficient concentration of analytes [64-66]. In contrast, one study found that two different stir speeds (250 and 400 rpm) had no effect on FC when evaluating the removal of methylene blue from water [67]. Although it was expected that higher stir speeds would result in greater f_{LOD} and signal enhancements in the current study, we found that extraction at lower speeds resulted in higher signal enhancements (12.3 for 1200 rpm, 11.4 for 1400 rpm and 10.6 for 1740 rpm). Careful

observation identified that vigorous stirring of the last of 500 μL of solution produced splashing, with higher stir speeds resulting in more splashing. It is likely that increased splashing decreased the efficiency of ICECLES during freezing of the last milliliter of the sample solution. It is likely that the final volume of solution is quite important to the overall extraction efficiency because the concentration stress is greatest as this part of the solution is frozen. Therefore, disruption of uniform stirring as the final volume of solution is frozen likely decreased the extraction efficiency.

The effect of freeze rate on the extraction efficiency was also evaluated by varying the coolant temperature during ICECLES. Most previous studies of FC, have shown that increasing the freeze rate decreases the extraction efficiency [64, 65, 67]. For example, in the FC of methylene blue from water, researchers found that concentration of methylene blue was more efficient at higher temperatures (-6 ^{o}C verses -24 ^{o}C) [67]. Similarly, Fujioka [65] and Miyawaki [64], found that extraction at lower freeze rates resulted in more concentrated solutions. Conversely, Gao et al. [72] found that FC for organic contaminant removal from petroleum effluent was not affected by the freezing temperature (-10 ^{o}C and -25 ^{o}C).

For the current study, variation in coolant temperature produced only minor differences in signal enhancement. Figure 3 shows the SBSE and ICECLES signals for side–by–side analysis of benzaldehyde for multiple freezing rates with no observable trend. Complete freezing was accomplished in 12, 6, 4, and 3 hours for -3, -5, -7 and -9 ^{o}C, respectively. Although freezing at -9 ^{o}C produced the shortest analysis time and resulted in the highest SE, it resulted in lower signals for both ICECLES and SBSE. This is likely because the contact time of the stir bar and the sample is reduced, which may not

allow time for the analyte to fully reach equilibrium between the stir bar and the aqueous solution. Although the optimized ICECLES freeze rate required 3.5-4 hours to complete the extraction, the sample preparation time can be reduced if some signal can be sacrificed (Figure 3). For comparison, typical extraction times for SBSE range from 30 min to four hours [73].

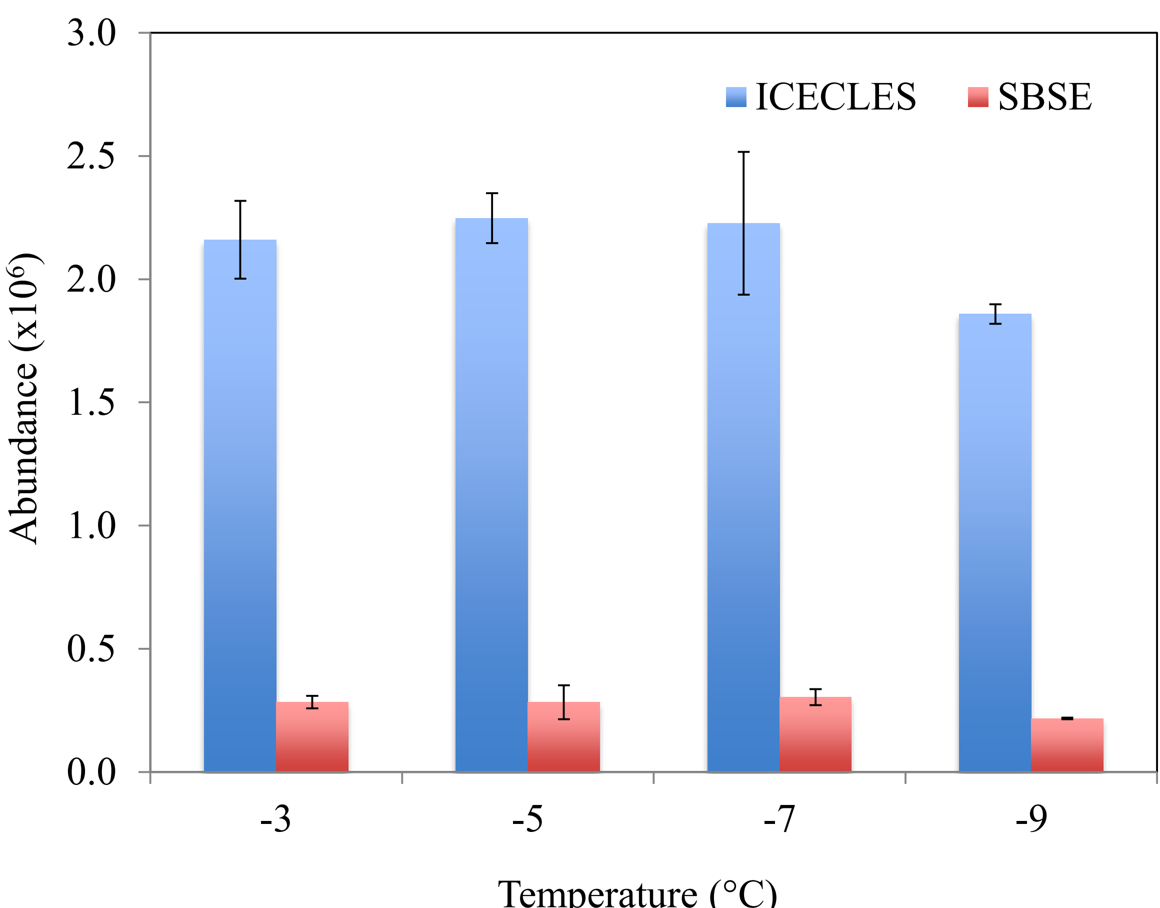


Figure 2.3. Effect of different coolant temperatures (i.e. freeze rates) on ICECLES for aqueous solution of 100 nM benzaldehyde. SBSE was evaluated using the same sample preparation time as ICECLES.

2.4.3. Effect of Initial Analyte Concentration

The initial concentration of the analyte has been previously shown to affect the efficiency of FC. For example, when investigating FC of glucose in a model solution, Miyawaki et al. [64] found that lower initial concentrations of solutes resulted in more efficient FC, and Fujioka et al. [65] found a similar result for desalination of ice. In contrast, during the use of FC for organic contaminant removal from a petroleum refinery secondary effluent, Gao et al. [72] reported that the initial concentration of the sample had no significant effect on the extraction efficiency.

For this study, we found that the initial concentration of benzaldehyde had minimal effect on ICECLES extraction efficiency and no trend was observed. The benzaldehyde concentrations tested (100, 200, 400, 600, 800, 2000 and 3000 nM) produced SEs of 9.5, 13, 12.8, 9.1, 10.4, 11.3 and 14.8, respectively.

2.4.4. Linear range, accuracy and precision

To determine the calibration behavior of compounds prepared via ICECLES, a benzaldehyde calibration curve was constructed in the range of 40-5000 nM and is shown in Figure 4. Analysis of the calibration standards, both weighted (1/x and $1/x^2$) and unweighted, indicted that a 1/x weighted fit was best able to describe the calibration data. Evaluation of Figure 2.4 shows that the calibration curve is clearly linear, producing a correlation coefficient (R^2) of >0.999. Accuracy and precision were determined by analysis of a low, medium, and high QC (75, 300, 1500 nM, respectively). The accuracy was 100 ± 15% and the precision was ≤ 16% RSD for the QCs. Even though the precision of the high QC was quite large (16% RSD), the worst precision of the other QCs and calibration standards was 7% RSD. Therefore, a more realistic precision is

likely in the range of 5-7% RSD for the implementation of ICECLES used in the current study.

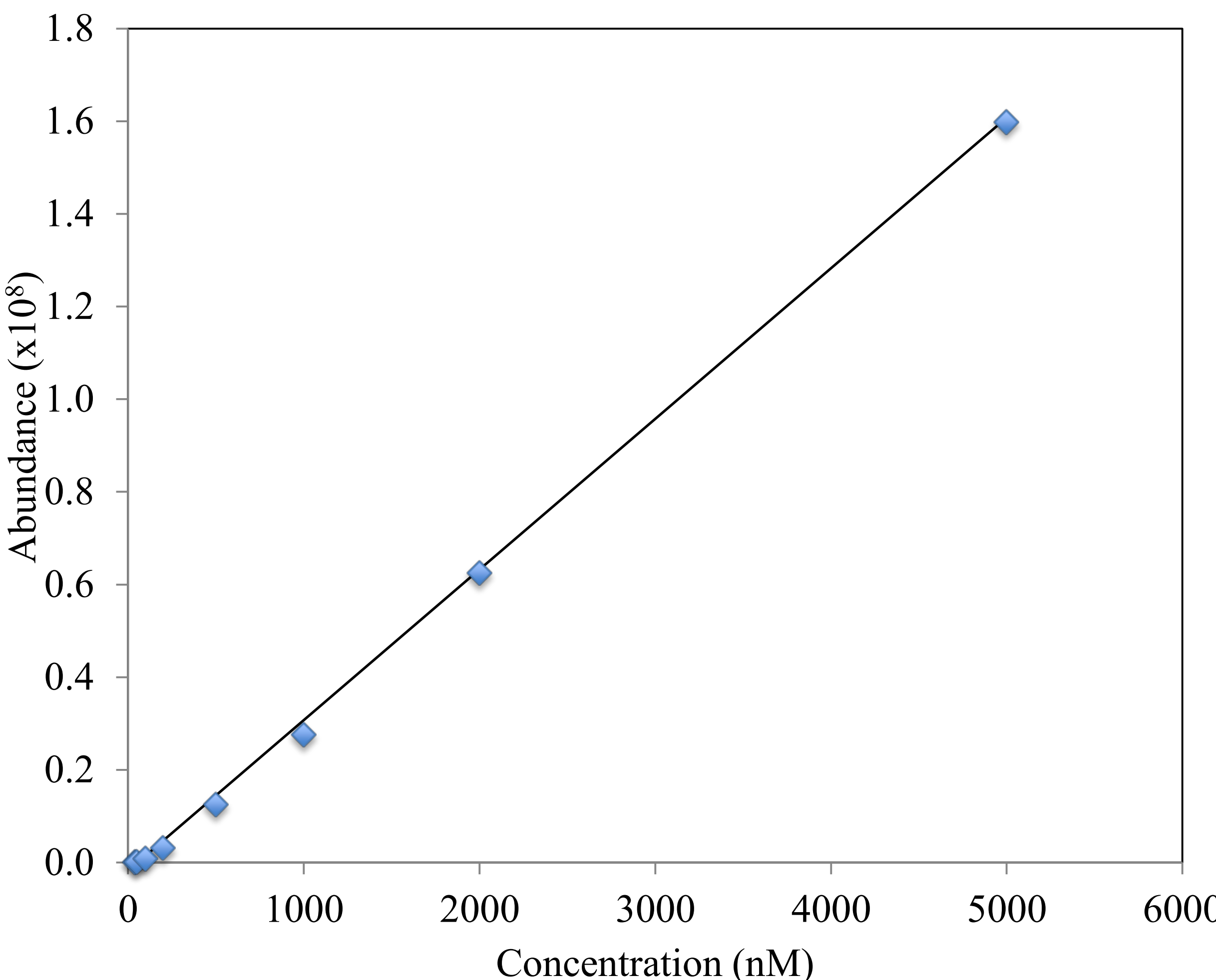


Figure 2.4. The calibration behavior of benzaldehyde (40-5000 nM) prepared via ICECLES. The calibration curve is clearly linear with a 1/x weighted fit producing an R^2 of > 0.999.

Table 2.2. The accuracy and precision of benzaldehyde QCs prepared via ICECLES.

Concentration (nM)	Precision (%RSD)	Accuracy
75	2.2%	100±3%
300	5.1%	100±10%
1500	16.0%	100±15%

2.4.5. Comparison of ICECLES and SBSE

Following optimization and evaluation of concentration effects, multiple analytes with varying K_{ow}s were investigated by both SBSE and ICECLES. Reported in Table 3, are the LODs (SBSE and ICECLES), LLOQ (SBSE only), SE and ratio of LODs (f_{LOD}) for each analyte tested. Figure 5 shows the signal enhancement plotted as function of log K_{ow}. As observed in Table 3 and Figure 5, ICECLES produced improved LODs and generated significant SEs compared with SBSE for each analyte. ICECLES sample preparation was clearly able to efficiently extract analytes at low concentration and performed better than SBSE for all analytes, but especially for more hydrophilic compounds (i.e., those with lower log K_{ow}). In fact, ICECLES was capable of producing signal enhancements of nearly 500 times SBSE. Furthermore, the LODs produced by ICECLES are much lower than SBSE, with more hydrophilic compounds again benefitting more than more hydrophobic compounds.

As expected, the hydrophobicity of an analyte (as quantified by log K_{ow}) was the most important property in determining the signal enhancement and extraction efficiency. The compound with the lowest log K_{ow}, 2-butanol (log $K_{ow} = 0.6$), produced the largest signal enhancement of 474. However, bromobenzene, which has the highest log K_{ow} among the compounds tested (log $K_{ow} = 2.9$), gave a SE of only 1.2 times. Because

approximately 90% of an analyte with a log K_{ow} of 3 can be extracted with SBSE alone, there is little to gain by using ICECLES on compounds with log K_{ow}s near or above this value. Considering this, one of the main advantages of the ICECLES over the SBSE is that ICECLES can be applied for compounds that are more hydrophilic.

There is also a limit to the utility of ICECLES for lower log K_{ow} compounds (at least for PDMS-coated stir bars). This is indicated by the increase in the absolute value of the LOD as the SE and f_{LOD} are greatly increasing. The increase in the LOD of hydrophilic compounds is caused by the unfavorable environment of the PDMS-coated stir bar for hydrophilic compounds. Therefore, as the log K_{ow} decreases, at some point the analyte will not sufficiently partition into the stir bar, no matter the concentration in the aqueous phase.

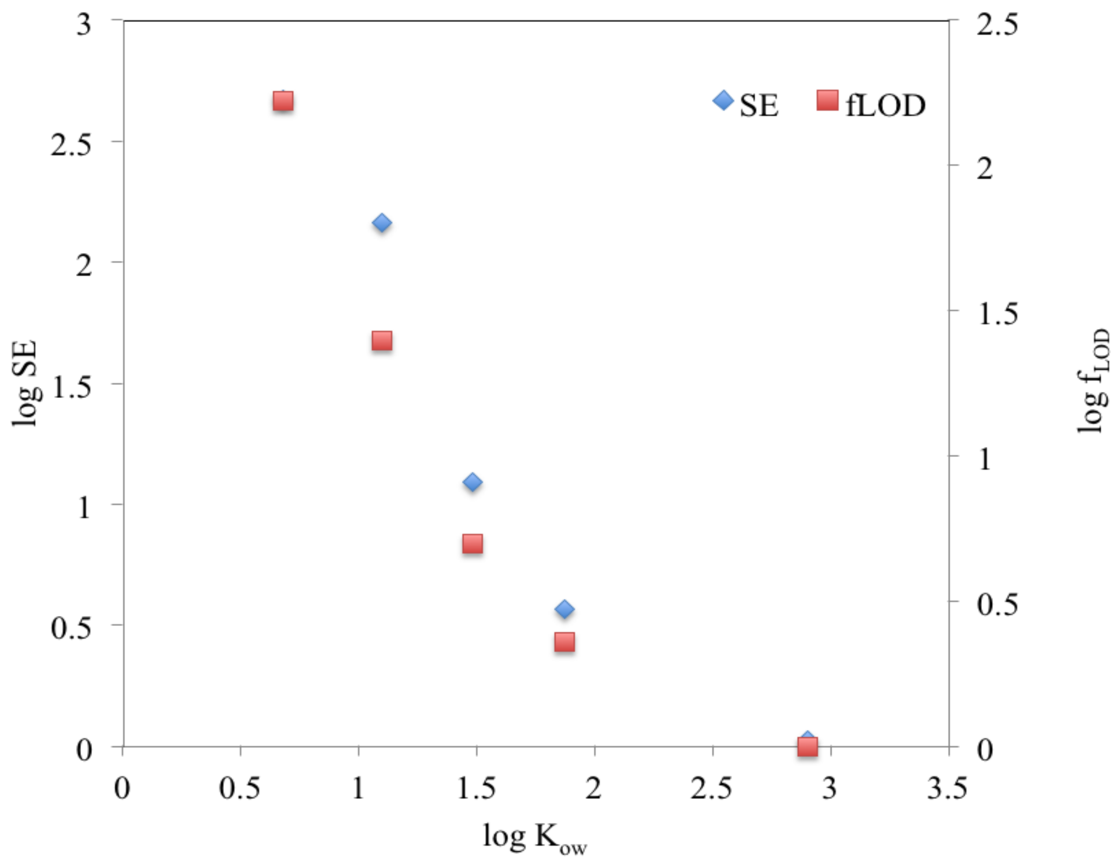


Figure 2.5. The performance of ICECLES relative to SBSE for compounds with log K_{ow}s ranging from 0.6 – 2.9. The behavior of signal enhancement (SE) and f_{LOD} are similar, with ICECLES performing better relative to SBSE as the K_{ow} decreases.

Table 2.3. Comparison of SBSE and ICECLES for a range of K_{ow}s.

Analytes	log K_{ow}	LLOQ for SBSE (nM)	LOD (nM)		SE	f_{LOD}
			SBSE	ICECLES		
2-butanol	0.61	140000	42000	250	474.9	168
Benzyl Alcohol	1.1	3300	1000	40	146.4	25
Benzaldehyde	1.48	333	100	20	12.3	5
Dimethyl trisulfide	1.87	23	7	3	3.7	2.3
Bromobenzene	2.9	2	0.6	0.4	1.2	1.5

2.5. Conclusion and Future work

Presented for the first time in this study, the highly complementary nature of FC and SBSE produced a novel analytical sample preparation technique called ICECLES, which is well−suited for trace analysis of water samples. ICECLES proved to be an effective sample preparation method for trace analysis, performing better relative to SBSE, especially for lower log K_{ow} compounds. As compared to SBSE, a maximum signal enhancement of around 500 times was observed for the lowest log K_{ow} analyte tested (0.61), and 1.2 times for an analyte with a log K_{ow} approaching 3. Moreover, ICECLES can be easily implemented and should be well-suited for analysis of trace-level concentrations, thermally labile compounds, more volatile compounds, and simultaneous analysis of compounds with a wide range of hydrophobicities. Although the implementation of ICECLES used for this study was shown to perform very well, the limited sample volume and long sample preparation times are areas for future improvement.

Chapter 3. Determination of triazine pesticides from aqueous sample using ICE Concentration Linked with Extractive Stirrer (ICECLES)

3.1. Abstract

A sensitive and simple analytical procedure used for the simultaneous determination of triazine pesticides was developed using ICECLES. ICECLES with thermal desorption gas chromatography mass spectroscopy (TD-GCMS) proved to be an effective method for multiple pesticides in water samples. Five triazine pesticides, with a range of log K_{ow}s, were tested using ICECLES. ICECLES generated signal enhancements of up to 227 times SBSE for metamitron (log K_{ow} = 0.83; the lowest of the compounds tested) and a signal enhancement of 2.5 times SBSE for atrazine (log K_{ow} = 2.4; the highest tested). Moreover, ICECLES lowered the LOD for all the triazines analytes tested. Overall, ICECLES provided excellent advantages for polar pesticides present at trace levels in source and treated drinking water samples.

3.2. Introduction

Contamination of environmental water resources is one of the major concerns for preservation and sustainability of the environment. One class of common contaminants is agricultural chemicals (pesticides or herbicides), which are introduced into water supplies from runoff (i.e., triazine, organophosphates and carbamates). Because of their extensive use in worldwide agricultural for production, these compounds represent an important and common class of pollution for environmental water sources [74]. One class of pesticides frequently used in the US for weed control, is the triazine group. Triazines are selective herbicides that have been used to control weeds for decades because their ability

to inhibit photosynthesis in plants [75]. For example, in 1992, around 69% of US land used for corn production was treated with atrazine [76]. Not only are triazines extensively used they, may persist in ground and surface water. According to recent studies, atrazine and simazine are the two main triazine pesticides detected in natured water sources [77]. Atrazine is a carcinogen [78] which causes DNA damage and endocrine disruption [76, 79]. Moreover, in 1998 to 2003, approximately 7 million people were exposed to atrazine in drinking water in the US [80]. Therefore, the Environmental Protection Agency (EPA) regulated atrazine at 200 ppb in drinking water based on its one-in-a-million cancer risk [77].

Because triazine pesticides are of concern, even at trace levels, their determination from environmental and drinking water sources requires advanced sample preparation techniques such as solid phase extraction (SPE), solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE) [75]. Even though these techniques can produce low detection limits, good reproducibility, and high recoveries [75], trace analysis of the triazine pesticides in environmental water source is difficult, especially for the more polar compounds in this class. For monitoring environmental water source contaminants and evaluation of the efficiency of water treatment techniques, analytical methods that are reliable and sensitive to trace level contamination are required.

ICECLES is a novel sample preparation technique, described in Chapter 2, that proved to be an effective technique for trace and ultratrace analysis of both nonpolar and more polar analytes. ICECLES is a sensitive and reproducible technique that is a combination of stir bar sorpitive extraction (SBSE) and freeze concentration (FC).

ICECLES has produced excellent signal enhancements and much lower LODs for lower polarity analytes. Therefore, the objective of this work was to determine triazine pesticides in environmental and drinking water samples at trace levels using ICECLES.

3.2. Experimental

3.2.1. Materials and Methods

All reagents were LCMS grade unless otherwise noted. The pure standard of desethlyatrazine (DEA), desosipropylatrazine (DIA), metribuzin (MTB) and metamitron (MTT) were purchased from Dr. Ehrenstorfer GmbH (Augsburs, Germany). Atrazine in acetone was purchased from Sigma-Aldrich (St. Louis, MO, USA). Purified water was obtained from a water PRO PS polisher (Labconco, Kansas City, KS, USA) at a resistivity of 18.2 MΩ-cm. Source and treated water samples were obtained from Brookings Municipal Utilities on March 18, 2016 at 2:30 p.m. Water before treatment was obtained on site from a ground water well 50 feet below the water surface and approximately 3 feet above the bottom of the well using a submersible sump pump. Treated water was treated using this process, aeration, coagulation (primary clarifier), recarbonation and filtration [81, 82].

3.2.2. Preparation of Standards

Standard solutions of each analyte, except atrazine, were prepared by directly diluting in water. Because of its low solubility in water, atrazine was prepared by diluting in water from a received standard in acetone. After preparation, stock solutions of MTT (8.6 mM), MTB (5.58 mM), DIA (3.58 mM), DEA (17 mM) and ATR (4.5 mM) were stored at 4 ^{o}C. Samples for each individual experiment were prepared by series dilution from a stock solution with purified water to the desired concentration. All samples and

standards were analyzed in triplicate unless otherwise indicated. When analyzing multiple triazine pesticides simultaneously, samples were prepared fresh by spiking the target compounds at 660, 66, 6.6, 13 and 1.3 μM for MTT, DIA, DEA, MTB and ATR, respectively.

ICECLES (described in chapter 2) was used to prepare water samples for this study. Briefly, each spiked or non-spiked water sample (10 mL) was added to a 24 mL glass vial. A SBSE stir bar with PDMS coating (Twister™, 10 mm with 0.5 mm thickness) was added into the vial, the vial was capped, and placed into a double-walled beaker. A small amount of methanol (1 mL) was added to the beaker to increase the thermal contact between the vial and the cooled beaker. Samples were extracted for 3.5-4 hours with a coolant temperature of -7 ^{o}C and a stir rate of 1200 rpm. The sample froze from the bottom of the vial to the top until the entire aqueous sample was frozen with the stir bar remaining stirring on the top of the ice surface. After extraction, the stir bar was magnetically removed using a clean Teflon-coated stir bar. The SBSE stir bar was then placed into a thermal desorption (TD) tube for further analysis.

Source drinking water (without treatment) was filtered four times using 0.2 μm membrane syringe filters prior to ICECLES. Note: A slightly higher freezing temperature (-5 ^{o}C) was used for ICECLES of drinking water samples because the impurities in the water, such as salts, interfered with the integrity of the ice crystal at faster freeze rates.

3.2.3. Lower Limit of Quantification, Signal Enhancement and Limit of Detection

The lower limit of quantification for SBSE was determined by evaluating multiple concentrations of each analyte and selecting the concentration that reproducibly produced a signal to noise ratio of 10, with noise measured as the peak-to-peak noise of the blank

over the elution period of the respective analyte. Utilizing the SBSE LLOQ concentration, ICECLES was performed in triplicate and the signal enhancement (SE) was calculated by dividing the average peak area of ICECLES by the respective average of peak area of SBSE for each analyte. The limit of detection (LOD) for both ICECLES and SBSE was estimated by the analysis of multiple concentrations of each compound below their LLOQ. The LOD was defined as the lowest analyte concentration that reproducibly produced a signal-to-noise ratio of 3. The LOD enhancement (f_{LOD}) was calculated by dividing the LOD for SBSE by the LOD for ICECLES under the same conditions, besides freezing the solvent for ICECLES (i.e., SBSE was completed on the same standard solution as ICECLES, but was performed in a vial directly placed on a stir plate and stirred for the entire ICECLES sample preparation time).

3.2.4. Instrumentation

SBSE stir bars were analyzed using thermal desorption gas chromatography mass spectroscopy (TD-GCMS). An Agilent Technologies 7890A with a 5975C inert XL electron ionization (EI)/chemical ionization (CI) mass selective detector (MSD) with Triple-Axis Detector and Multipurpose-Sampler (MPS) was used. Desorption of the analyte from the stir bar was carried out in splitless mode into a cooled injection system (CIS) liner. The thermal desorption temperature was initially held at 60 ^{o}C (for 0.20 min), and then quickly increased (720 ^{o}C/min) to 250 ^{o}C, where this temperature was held for 1 min. After the desorption step, the analyte was collected into the CIS at 30 ^{o}C. The analyte was then transferred into the GC column and separated on an HP-5MS capillary column (30 m x 250 μM x 0.25 μM) with nitrogen as the carrier gas at a flow rate of 1 mL/min and a pressure of 5.565 psi. The GC temperature gradient was optimized for each

analyte and is shown in Table 3.1, with the run time for each analyte also listed. When analyzing multiple triazine pesticides simultaneously, the oven gradient was initiated at 30 ºC (1 min) and then ramped at 60 ºC/min to 160 ºC (held for 1 min) and increased at 120 ºC/min to 280 ºC for 5 min. For all GC-MS methods, the MS source and MS quadruple temperatures were 280 ºC and 150 ºC, respectively. The EI source was 70 eV energy. The fragment ions monitored for identification and quantification are also shown in Table 3.1.

Table 3.1. Important GC-MS parameters for the five compounds used in this study to evaluate ICECLES sample preparation.

Compound	Thermal Gradient for GC			Run-Time (min)	Elution-Time (min)	Ion Mass (m/z)	
	Initial	Ramp 1	Ramp 2			Quantification	Identification
Atrazine (ATR)	30 ^{o}C (0.5 min)	30 ^{o}C/min to 160 ^{o}C (0 min)	120 ^{o}C/min to 280 ^{o}C (3 min)	8.8	7.0	215	200
Atrazine desethyl (DEA)	30 ^{o}C (0.5 min)	30 ^{o}C/min to 160 ^{o}C (0 min)	120 ^{o}C/min to 280 ^{o}C (2 min)	7.8	6.8	188	172
Atrazine desosipropyl (DIA)	30 ^{o}C (1 min)	30 ^{o}C/min to 120 ^{o}C (0 min)	120 ^{o}C/min to 250 ^{o}C (1 min)	8.1	6.8	158	173
Metribuzin (MTB)	30 ^{o}C (1 min)	90 ^{o}C/min to 170 ^{o}C (1 min)	120 ^{o}C/min to 280 ^{o}C (5 min)	9.4	6.1	214	198
Metamitron (MTT)	30 ^{o}C (1 min)	60 ^{o}C/min to 160 ^{o}C (0 min)	120 ^{o}C/min to 280 ^{o}C (5 min)	8.6	6.9	104	202

3.3. Results and Discussion

3.3.1. Comparison ICECLES and SBSE

ICECLES was created to improve extraction efficiencies of analytes compared to SBSE. In this study, triazine analytes with varying log K_{ow}s were investigated by both SBSE and ICECLES. SBSE and ICECLES LODs, SBSE LLOQs, SEs and f_{LOD}s for each analyte are reported in Table 3.2. Additionally, the SE and f_{LOD} are plotted together in Figure 3.1. ICECLES clearly performed better than SBSE, both decreasing the LOD and enhancing the signal for each triazine pesticide tested (Figure 3.1.). ICECLES was more effective for hydrophilic analytes (i.e. those with lower log K_{ow}s). In this work, ICECLES generated signal enhancements up to 227 times for metamitron (lowest log K_{ow} analyte tested), and approximately 2.5 for atrazine (highest log K_{ow} analyte tested). The SE trend follows: metamitron > desiopropyl-atrazine > desethyl-atrazine > metribuzin > atrazine. ICECLES was also capable of decreasing the LOD to over 100x that of SBSE. Although the SE and f_{LOD} are the lowest for ATR, it still has the lowest LOD for both SBSE and ICECLES it is most stable in the PDMS coating of the stir bar.

Following analysis of each individual analyte, a single TD-GCMS method was then developed for simultaneous analysis of all analytes. This method was effective at analyzing all five analytes over 10 minutes. Subsequently, SBSE and ICECLES were used to prepare aqueous sample with all five analytes present. The resulting chromatograms are shown in Figure 3.2. which, illustrates that DEA and DIA eluted at the same retention time (5.8 min), ATR, MTB and MTT eluted at 6.2, 6.6 and 7.8 min respectively. The peak shapes for (Atrazine and Metribuzin) were sharp, but the other peaks were not sharp. All peaks showed significant tailing. Only atrazine and metribuzin

were detected using SBSE, all the five pesticides were clearly detected using ICECLES. As expected, ICECLES was more effective than SBSE for polar triazine pesticides (metamitron, DIA and DEA), but it was also more effective for the analysis of ATR and MTB, as seen by two increased signal strengths for these two analytes.

Overall, ICECLES sample preparation and analysis was simple, reproducible and effective. Figure 3.2 indicates the power of ICECLES for simultaneously detecting analytes with a wide range of log K_{ow}s. Therefore, in situations were multiple pesticides must be quantified simultaneously, ICECLES should be considered, especially if some of pesticides to be analyzed have relatively low K_{ow}s (i.e. log K_{ow}s ≤ 2.5.

Table 3.2. Comparison of SBSE and ICECLES for a range of K_{ow}s pesticides.

Analyte	log K_{ow}	LLOQ for SBSE (nM)	LOD (nM)		SE	f_{LOD}
			SBSE	ICECLES		
Metamitron (MTT)	0.83	250000	60000	1000	227	60
Atrazine desosipropyl (DIA)	1.2	150000	50000	500	137	100
Atrazine desethyl (DEA)	1.51	6500	2000	50	16	40
Metribuzin (MTB)	1.71	830	250	40	8	6.65
Atrazine (ATR)	2.4	200	80	20	2.5	4

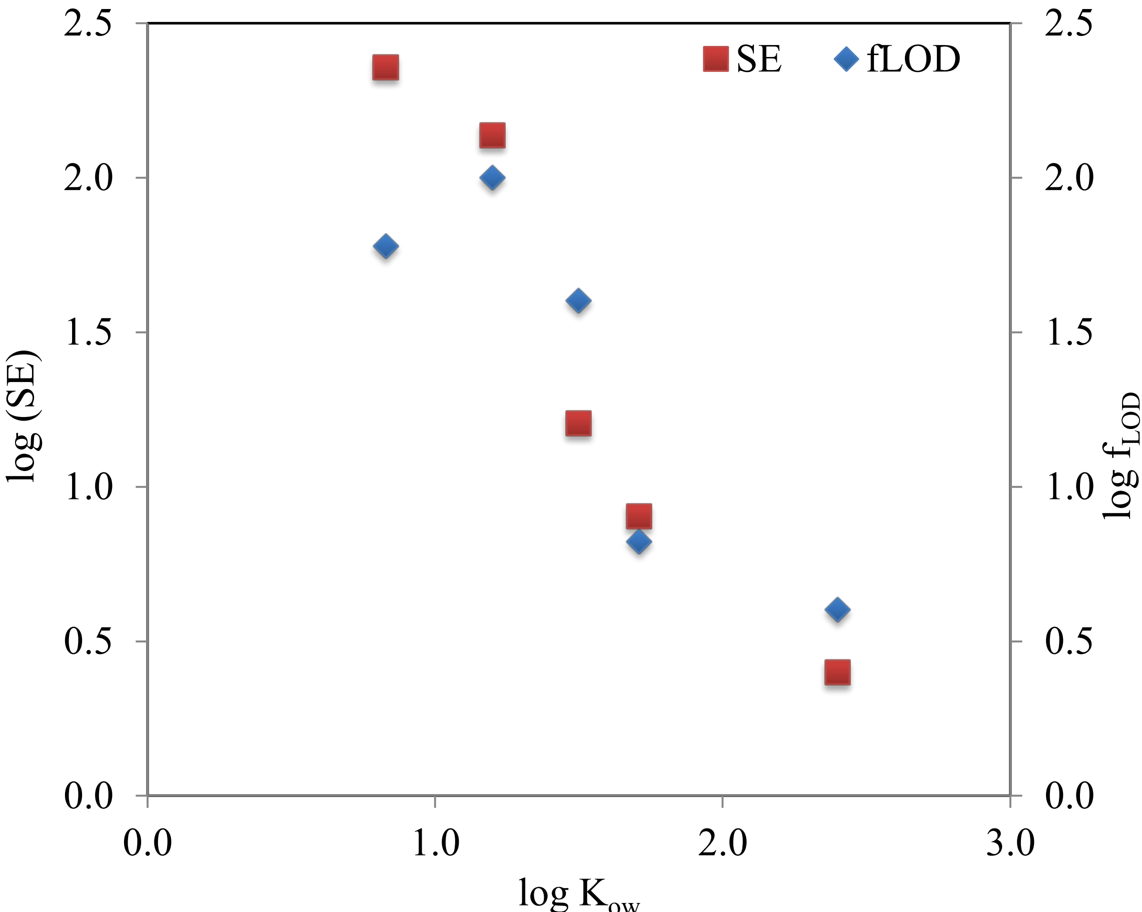


Figure 3.1. The signal enhancement and LOD ratio for a wide range of log K_{ow} (0.83-2.4) triazine pesticides. The behavior of signal enhancement (SE) and f_{LOD} are relatively similar, with ICECLES performing better relative to SBSE as the K_{ow} decreases.

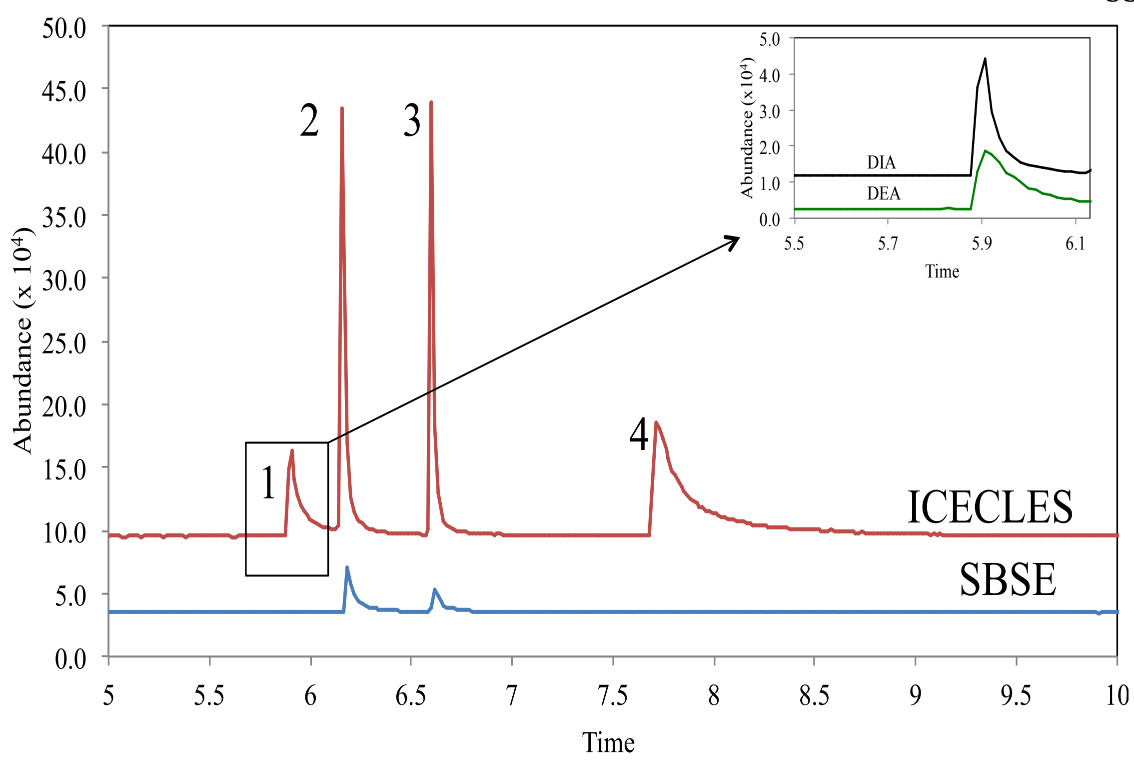
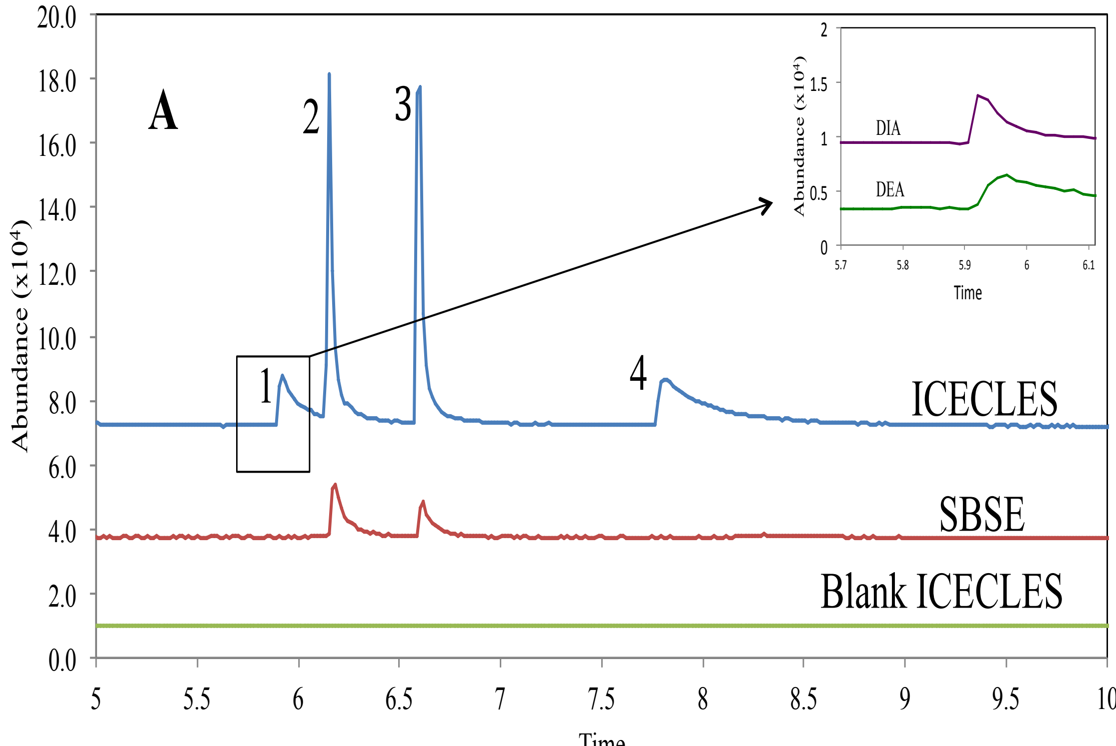


Figure 3.2. Pesticide chromatograms for ICECLES and SBSE. Chromatographic peaks are labeled as ICECLES 1) deethylatrazine and deisopropylatrazine, 2) atrazine, 3) metribuzin and 4) metamitron.

3.3.3. Analysis of pesticides in Source and Treated Drinking Real Water

After a single method was created to analyze the pesticides, drinking water from Brookings Municipal Utilities (Brookings, SD) was tested before and after treatment. Each of the triazine pesticides were not detected in the drinking water (before treatment and after treatment) likely because the triazine pesticides degraded over the period. The triazine pesticides (especially atrazine) uses only in spring and summer [83] and our analyzing time completed in the winter seasons.

To ensure the method could detect the five analytes from these matrices, the water before and after treatment was spiked with the triazine pesticides. Figure 3.3 A shows spiked source water and B shows spiked treated water prepared with both ICECLES and SBSE. The Figure shows that ICECLES was capable of detecting all the pesticides, while only atrazine and metribuzin were detected using SBSE. The data from Figure 3.3 (A and B) illustrate that ICECLES can extract analytes from both source and treated water with no differences observed between the extraction efficiency of these pesticides. The data in Figure 3.3 indicates that ICECLES is an effective technique for simultaneously analyzing multiple pesticides with a wide range of polarities from drinking water.



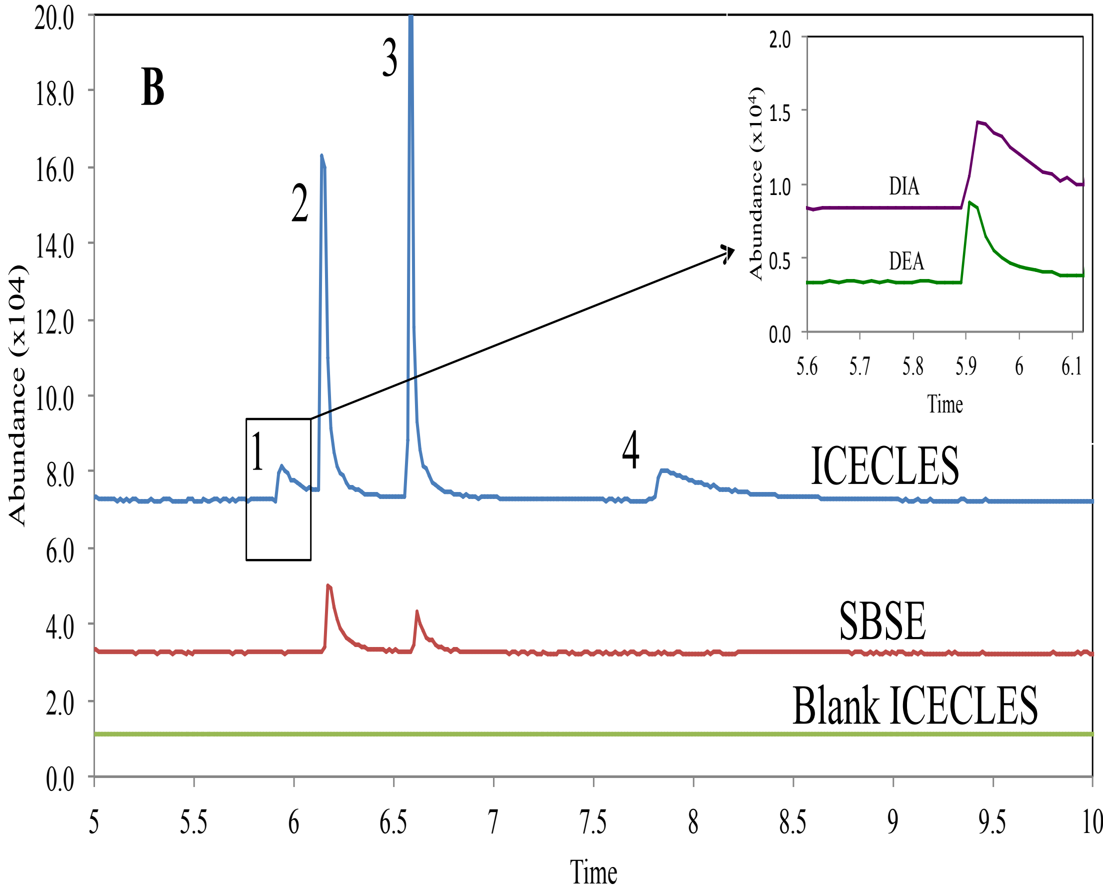


Figure 3.3. Chromatograms for ICECLES and SBSE for application of spiked drinking water before (A) and after (B) treatment with blank for each water for ICECLES. Chromatographic peaks are labeled 1) deethyl-atrazine and deisopropyl-atrazine, 2) atrazine, 3) metribuzin and 4) metamitron.

3.4. Conclusion

A simple analytical technique for determination of triazine pesticides in aqueous samples was developed. ICECLES was sensitive and reproducible preconcentration technique used for triazine pesticides. ICECLES proved more advantageous to SBSE, the lower the log K_{ow} of the pesticides. ICECLES was more effective for simultaneously

analyzing for multiple pesticides in water samples compared to SBSE, where ICECLES was capable of detecting more the polar analytes.

Chapter 4: Conclusion and Future Work

4.1. Conclusion

The novel technique developed in this work combined complementary nature of two advanced techniques: SBSE and FC. ICECLES was an effective technique for trace and ultratrace especially suited for volatile and thermally unstable compounds. ICECLES shows excellent extraction efficiency for lower log K_{ow} compounds as compare to SBSE. ICECLES produced signal enhancements of up to 474 times SBSE for lowest log K_{ow} analyte tested. ICECLES was capable of decreasing the LODs over 100x compared to SBSE. Although SEs and f_{LOD}s increase with decreasing K_{ow}, higher log K_{ow} analytes still produced lower ICECLES LODs because the hydrophobicity of these analytes increase their affinity for the PDMS coating.

This study also proved that ICECLES was an efficient technique for the determination of triazine pesticides with a wide range of log K_{ow}s (0.83-2.4) from aqueous samples. ICECLES was able to generate signal enhancements up to 227 for metamitron (i.e., the lowest log K_{ow} analyte) and 2.5 for atrazine (i.e., the highest log K_{ow}). Moreover, ICECLES was an effective sample preparation method for the analysis of multiple pesticides simultaneously from source and treated drinking water samples. ICECLES was especially favorable for simultaneous analysis of analytes with a wide range of log K_{ow}s and should be considered a favorable method for the simultaneous analysis of analytes with a wide range of log k_{ow}.

Even though ICECLES proved to have a numerous advantages, there are also limitations, such as the limited sample volume and the relatively and potentially long extraction times necessary for the implementation of ICECLES used in this study.

4.2. Future Work

The development of a more sophisticated ICECLES apparatus should be the area of focus to solve the two limitations of sample preparation via ICECLES.. Moreover, optimization of the chromatography and validation of the multiple pesticides should be completed to determine the linear range for each analyte, precision and accuracy.

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