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Exploration of Vase as an Extraction Method for Emerging Disinfection By-products and Acidic Methanol as a Safe and Effective Derivatization Method for Haloacids

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EXPLORATION OF VASE AS AN EXTRACTION METHOD FOR EMERGING DISINFECTION BY-PRODUCTS AND ACIDIC METHANOL AS A SAFE AND EFFECTIVE DERIVATIZATION METHOD FOR HALOACIDS

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DEDICATION

I dedicate this masters thesis to my family. I want to thank my Dad (Lee M. Kilpatrick), my Mom (Debbie M. Kilpatrick), and my sister (Morgan S. Kilpatrick) especially for making me the woman I am today. Without your long goodbye waves, jokes, and warm hugs I would not have been able to do this.

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ABSTRACT

This project aims to create an environmentally friendly, robust, and efficient extraction and preconcentration method in both the detection and quantification of various disinfection by-products (DBPs). Vacuum assisted sorbent extraction (VASE) is a newly developed method in the extraction of both volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs). This extraction method is paired with a Thermo Scientific TSQ Quantum Triple Quadrupole GC-MS system for the quantification of DBPs. VASE outperforms current DBP extraction methods, with liquid-liquid extraction (LLE) being the most used alternative, because of its minimal use of solvent, high level of automation, and over-all ease of use. A series of methods is shown for representative compounds from multiple DBP classes. In addition to this, a singular method for the thermal desorption of 44 compounds has been established. In-vial derivatization of the haloacetahldehyes (HALs) is also explored as a novel approach to derivatization for the third largest by weight DBP class. Quantification and qualification of DBPs have been seen as low as 1 ng/L (ppb) in the early stages of method development, with lower detection limits expected with further optimization. Method development explores pH adjustment, agitation speed variation, ionic strength, 2-stage desorption, and in-vial derivatization. Real application of drinking-water and urine samples is also explored.

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LIST OF ABBREVIATIONS

DBP ..... disinfection by-product

EPA..... Environmental Protection Agency

GC-MS ..... gas-chromatography mass-spectrometry

HAAs .....the class of haloacetic acids

HALs ..... the class of haloacetaldehydes

HAMs .....the class of haloacetamides

HANs ..... the class of haloacetonitriles

HNMs ..... the class of halonitromethanes

IAAs .....the class of iodoacetic acids

I-THMs ..... the class of iodo-trihalomethanes

MTBE ..... methyl tert-butyl ether

MeOH.....methanol

SVOC ..... semi-volatile organic compounds

THMs .....the class of trihalomethanes

VASE ..... vacuum assisted sorbent extraction

VOC .....volatile organic compounds

CHAPTER 1: INTRODUCTION: BACKGROUND OF DRINKING WATER AND DISINFECTION BY-PRODUCTS

Drinking water disinfection was created to control microbial pathogens to control the waterborne diseases they cause. Unfortunately, in the process, disinfection by-products (DBPs) are formed. DBPs form from the reactions between disinfectants (HOCl, NH_2Cl, ClO_2, O_3, and UV) and natural organic matter (NOM). DBPs are always present in drinking water at trace levels (µg/L).[1-5] Long-term exposure to DBPs has been linked to several health effects including bladder cancer, colon cancer, and various adverse birth outcomes.[6-15] Because DBPs are linked to negative health effects, it is extremely important to monitor the levels at which they reside in drinking water.

More than 700 DBPs have been identified in disinfected ground and surface waters.[5, 17, 18] As of currently, there are only 12 DBPs that have regulations and guidelines world-wide; these compounds include trihalomethanes (THMs) and haloacetic acids (HAAs).[2-5, 16] Other DBP classes include iodo-trihalomethanes (I-THMs), haloacetonitriles (HANs), haloacetaldehyes (HALs), halonitromethanes (HNMs), haloketones (HKs), haloacetamides (HAMs), and iodoacetic acids (IAAs). The following chapters explore methods used to optimize the detection of DBPs across the board. Optimization of these compounds is done with gas-chromatography mass-spectrometry (GC-MS).

In Chapter 2, the HAA and IAA class of DBPs is the focus. These classes of DBPs are not detected at traces levels unless derivatized prior to GC-MS analysis. Derivatization is utilized in order to increase the volatility and lower the polarity of haloacids, so they are seen with GC-MS. Two derivatization methods are explored and directly compared in the analysis of 13 haloacids. These two methods are acidic methanol and diazomethane generation. Diazomethane generation can be dangerous and has associated health risks, and while acidic methanol is much safer, it has many more steps and has potential to introduce a larger source of error. These two methods have not previously been directly compared for all 13 haloacids (9 HAAs and 4 IAAs) and detected with a selected ion monitoring (SIM) method. In addition to comparing the 2 derivatization methods, a new SIM method was optimized for even lower detection of these compounds than seen before on a single quadrupole-MS.

Chapter 3 dives deep into headspace extraction of 50 unregulated DBPs. These compounds include the HANs, I-THMS, HALs, HNMs, HAMs, HKs, and a small list of thermally sensitive compounds called the “problem”-list. One method was developed to detect 44 of the 50 unregulated DBPs explored, while the 6 HALs require an in-vial derivatization and separate detection method as of now. Parameters explored include pH adjustment, ionic strength (salt addition), extraction temperature, and thermal desorption parameters. In addition to optimization of methods, the extraction of iodo-THMs was performed in drinking water and urine. The addition of headspace extraction with VASE shows promise for record breaking detection limits and omission of matrix effects in wastewater, urine, and others.

The focus of this thesis is improving the monitoring of toxic DBPs to lower human health effects and environmental effects caused by these compounds. In addition to that, the methods explored in chapters 2 and 3 aid in the scientist's ability to reproduce results and omit error. By working through these projects, it is clear that DBP analysis can be further improved. However, with the addition of a direct comparison of diazomethane and acidic methanol and a study of VASE as a headspace extraction method, we may be one step closer to monitoring these compounds more easily and effectively.

Table 1.1. A list of commonly analyzed DBPs

Class	**DBP**	**MW (g/mol)**
Iodo-Trihalomethanes	Triiodomethane (TIM) (Iodoform)	393.7
	Dichloroiodomethane (DCIM)	210.8
	Dibromoiodomethane (DBIM)	299.7
	Chlorodiiodomethane (CDIM)	302.3
	Bromodiiodomethane (BDIM)	346.7
	Bromochloroiodomethane (BCIM)	255.3
Regulated Trihalomethanes	Chloroform (TCM)	119.4
	Bromodichloromethane (BDCM)	163.8
	Dibromochloromethane (DBCM)	208.3
	Bromoform (TBM)	252.7
Regulated Haloacetic Acids	Chloroacetic acid (CAA)	94.5
	Bromoacetic acid (BAA)	139.0
	Dichloroacetic acid (DCAA)	128.9
	Bromochloroacetic acid (BCAA)	173.4
	Dibromoacetic acid (DBAA)	217.9
	Trichloroacetic acid (TCAA)	163.4
	Bromodichloroacetic acid (BDCAA)	207.8
	Dibromochloroacetic acid (DBCAA)	252.3
	Tribromoacetic acid (TBAA)	296.7
Haloketones	Chloropropanone (CP)	92.5

	1,3-Dichloropropanone (13DCP)	127.0
	1,1-Dibromopropanone (11DBP)	215.9
	1,1,3-Trichloropropanone (113TCP)	161.4
	1-Bromo-1,1-dichloropropanone (1B11DCP)	205.9
	1,1,3,3-Tetrabromopropanone (1133TeBP)	373.7
	1,1,1-Trichloropropanone (111TCP)	161.4
	1,1-Dichloropropanone (11DCP)	127.0
	1,1,3,3-Tetrachloropropanone (1133TeCP)	195.9
Iodoacetic Acids	Chloroiodoacetic Acid (CIAA)	220.0
	Bromoiodoacetic Acid (BIAA)	264.0
	Iodoacetic Acid (IAA)	186.0
	Diiodoacetic Acid (DIAA)	311.9
Haloacetonitriles	Chloroacetonitrile (CAN)	75.5
	Bromoacetonitrile (BAN)	120.0
	Iodoacetonitrile (IAN)	167.0
	Dichloroacetonitrile (DCAN)	109.9
	Bromochloroacetonitrile (BCAN)	154.4
	Dibromoacetonitrile (DBAN)	198.8
	Trichloroacetonitrile (TCAN)	144.4
	Bromodichloroacetonitrile (BDCAN)	188.8
	Dibromochloroacetonitrile (DBCAN)	233.3
	Tribromoacetonitrile (TBAN)	274.7
Halonitromethanes	Dichloronitromethane (DCNM)	129.9
	Bromochloronitromethane (BCNM)	174.4
	Dibromonitromethane (DBNM)	218.8
	Trichloronitromethane (TCNM)	164.4
	Tribromonitromethane (TBNM)	297.7
	Bromodichloronitromethane (BDCNM)	208.8
	Dibromochloronitromethane (DBCNM)	253.3
Haloaldehydes	Trichloroacetaldehyde (chloral) (TCAL)	147.4
	Bromodichloroacetaldehyde (BDCAL)	191.8
	Dibromochloroacetaldehyde (DBCAL)	236.0
	Tribromoacetaldehyde (TBAL)	280.7
	Dichloroacetaldehyde (DCAL)	112.9
	Bromochloroacetaldehyde (BCAL)	156.0

	Dibromoacetaldehyde (DBAL)	201.8
Haloamides	Chloroacetamide (CAM)	93.5
	Bromoacetamide (BAM)	138.0
	Iodoacetamide (IAM)	185.0
	Dichloroacetamide (DCAM)	128.0
	Bromochloroacetamide (BCAM)	172.0
	Dibromoacetamide (DBAM)	216.9
	Trichloroacetamide (TCAM)	162.4
	Bromodichloroacetamide (BDCAM)	206.0
	Dibromochloroacetamide (DBCAM)	251.0
	Tribromoacetamide (TBAM)	296.0
	Bromoiodoacetamide (BIAM)	263.0
	Chloroiodoacetamide (CIAM)	219.0
	Diiodoacetamide (DIAM)	311.0

CHAPTER 2: THE IMPORTANCE OF IAAS AND HAAS AND THE COMPARISON OF ACIDIC METHANOL TO DIAZOMETHANE DERIVATIZATION

2.1 Introduction & Background

The U.S. Environmental Protection Agency (EPA) has listed 5 haloacetic acids (HAA5) in drinking water regulation levels. These include monochloroacetic acid (CAA), monobromoacetic acid (BAA), dichloroacetic acid (DCAA), dibromoacetic acid (DBAA), trichloroacetic acid (TCAA). In addition to these regulated HAAs, there are 4 more that are unregulated; these include tribromoacetic acid (TBAA), bromodichloroacetic acid (BDCAA), bromochloroacetic acid (BCAA), and chlorodibromoacetic acid (CDBAA). In addition to the 9 haloacetic acids, the iodo-acetic acids are becoming of interest due to their potential toxicity.[21] Figure 2.1 shows the 9 haloacetic acids and 4 iodoacetic acids that are the focus of this study.

Haloacetic Acids and Iodoacetic Acids

The haloacetic acids (HAA9) are a very important class of disinfection by-products (DBPs) due to their high presence in drinking water; they represent the largest fraction of non-volatile DBPs.[25] In addition to this, the two most prevalent HAAs in drinking water are DCAA and TCAA[26] unless the source water contains extremely high levels of bromine—in that case the brominated DCBAA and BCAA are most prevalent.[22] This is

because bromine reacts much faster (close to 10x) with natural organic matter (NOM) than chlorine does.[23] The US EPA maximum contamination limits (MCLs) are set at 0.06

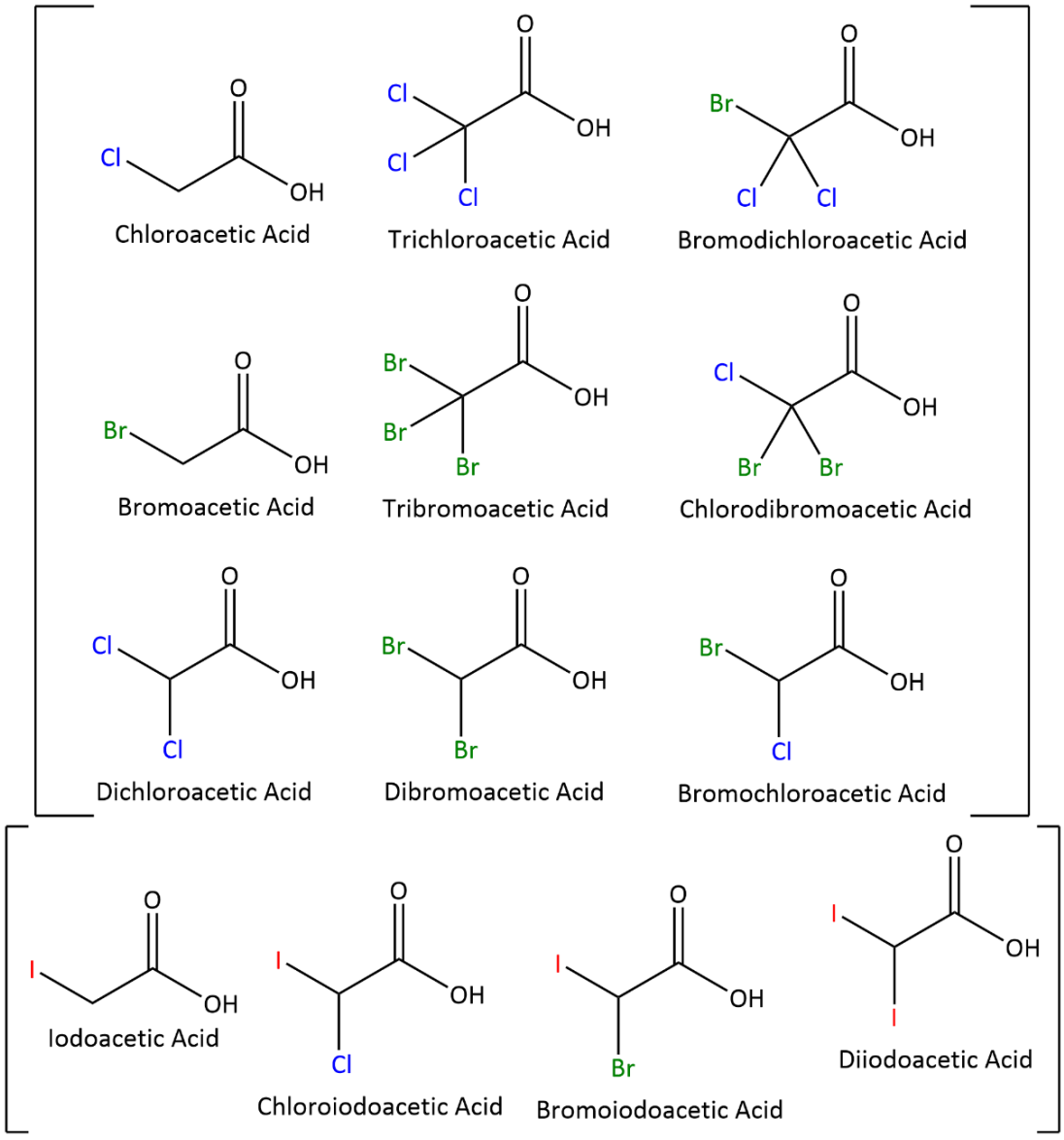


Figure 2.1. The underivatized structures of the 9 HAAs and 4 IAAs studied in this project

mg/L for the total sum of CAA, BAA, DCAA, DBAA, and TCAA.[24] This is because many have potential to be toxic, and in addition to this, many of them are considered possible carcinogens.[1]

The World Health Organization (WHO) also sets guidelines for 3 of these compounds: CAAs limit is 20 µg/L, DCAAs limit is 50 µg/L, and TCAAs limit is 20 µg/L.[27] Although the HAA5 compounds are considered of concern and are regulated (except in Europe[1]), the other 4 HAAs and the 4 IAAs are not regulated at all.

Iodoacetic acids form when the source water of a drinking water treatment plant (DWTP) contains high levels of iodide.[20] In 2002, the 4 IAAs shown in figure 2.1 were identified for the first time in a nation-wide DBP occurrence study.[43,25] Formation of these compounds is largely due to a change in disinfectant called chloramine. The change of DWTP disinfectant is because the regulated DBPs form at much lower levels when chloramine is used in place of (or in addition to) chlorine.[1] Although the regulated THM4 and HAA9 DBPs are seen at much lower levels, the use of chloramine results in different, and often more toxic, DBP formation.[21] In Plewa's study of chlorinated DBPs, brominated DBPs, and iodinated DBPs, it was found that iodo-DBPs are the most toxic with bromo- following behind and chloro- being the least toxic.[29,46-49] The reaction pathways of iodide and hypochlorous acid and iodide with chloramine show the two different reaction pathways with the use of the 2 different disinfectants. Figure 2.2 shows these reaction pathways. In the case of iodide and hypochlorous acid (chlorine disinfectant), the further reaction of HOI to iodite and iodate are extremely fast—this means that most of the naturally present iodide is converted very quickly to a non-toxic and very stable form (iodate). For the case of iodide and chloramine, the reaction is much slower (not kinetically favored), which leads to iodo-DBP formation.[28]

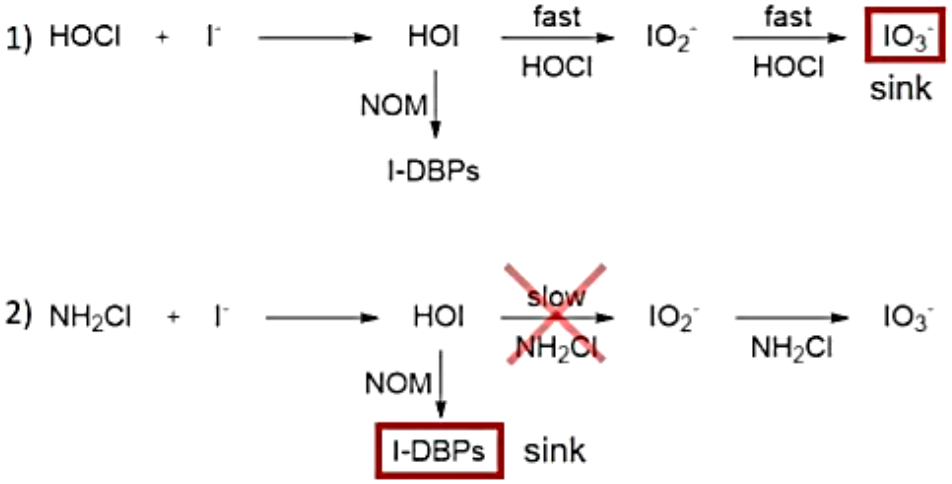


Figure 2.2. The reaction of iodide with hypochlorous acid (1) and chloramine (2).[20, 28]

Unfortunately, there are no iodo-DBPs regulated as of now, although they are more toxic than brominated or chlorinated DBPs.[29] Furthermore, this study looks specifically at the haloacids, and IAA is considered to be the most genotoxic DBP of all HAAs and IAAs identified.[21]

Derivatization: Why is it important?

As discussed in the previous paragraphs, these DBPs are extremely important to detect and analyze in drinking water. For analysis, these compounds are not detectable by GC-MS or GC-MS/MS without derivatization. This is because the haloacids are extremely acidic with pKa values between 0.5 and 3[31], so they are almost completely ionized during drinking water conditions. Even when the compounds are protonated, they are extremely polar, water soluble, and ultimately have very little volatility. The goal of derivatization is to add a functional group that will increase the volatility and

lower the polarity; this allows them to be detectable by GC. The two methods of derivatization used mostly include diazomethane and acidic methanol.

The diazomethane derivatization method works to convert a functional group into a methyl-ester functional group to the analyte derivatized, thus increasing the volatility of the compound present. Often, the analytes chosen for this type of derivatization include carboxylic acids, phenols, and alcohols.[32-34] This derivatization is called a methylation, and it is well known to be highly effective and rather quick.[32] Although this is the case, the use of diazomethane can be extremely dangerous because diazomethane is carcinogenic and can be explosive.[35,36] In addition to the health risks involved with use of diazomethane derivatization, it also provides low yields for the methylation of brominated tri-haloacetic acids (TBAA, BDCAA, CDBAA).[32]

Acidic methanol derivatization works as an acid-catalyzed Fischer esterification. The acidic methanol derivatization is also much safer than a diazomethane derivatization, however, it requires a molar excess of methanol and high temperatures due to the equilibrium reaction.[25, 32]

There are various standard operating procedures (SOPs) for the analysis of haloacetic acids provided by the U.S. EPA. For the use of diazomethane, U.S. EPA provides the SOP RSB 010.1[37], and for the use of acidic methanol, U.S. EPA provides the regulatory methods 552.1,[38] 552.2,[39] and 552.3[26]. The 3 methods for acidic methanol all explore different extraction techniques. The two acidic methanol methods that work best with drinking water analysis are EPA methods 552.2 and 552.3; these methods are used as regulatory methods for monitoring HAA5. Both EPA methods use a liquid-liquid

extraction followed by a derivatization step prior to gas chromatography-electron capture detection (GC-ECD) analysis. All three appropriate methods for drinking water analysis include 5 regulated HAAs (CAA, BAA, DCAA, DBAA, TCAA) and one unregulated HAA (BCAA), while the EPA methods 552.2 and 552.3 include all 9 non-iodinated HAAs. In order to optimize for selectivity and sensitivity, a method using gas chromatography coupled to triple quadrupole mass spectrometry (GC-MS/MS) in the multiple reaction monitoring (MRM) mode was developed by Li *et. al.*[31] for the analysis of HAA9 and IAA. A method was developed using a combination of all of these previously established methods for the analysis of all 9 HAAs and all 4 IAAs with GC-MS in selected ion monitoring (SIM) mode in order to compare the detection limits and extraction efficiencies of both diazomethane derivatization and acidic methanol derivatization.

Objectives

This project aims to provide a direct comparison between acidic methanol derivatization for HAAs and IAAs with the diazomethane derivatization currently used in this lab. The question posed: can acidic methanol, a much less toxic derivatizing agent, be an appropriate replacement for diazomethane in the derivatization of HAAs and IAAs? Overall, the goal is to determine if acidic methanol can be used as a safer compromise to diazomethane and to determine if the limit of detection is comparable for this method. In order to complete this task, an acidic methanol procedure was optimized by Jennifer Mueller in 2015[20], a visiting student from Germany. The method is in combination with GC-MS/MS and GC-MS detection which was developed, optimized,

and applied for the 9 HAAs and 4 IAAs. The final goal was to compare the derivatization efficiency of these 2 methods, and the limit of detection for the processes was analyzed.

2.2 Materials & Methods

Solutions:

Standards were purchased from multiple distributors based on availability. The nine haloacetic acids (chloroacetic acid- CAA, bromoacetic acid- BAA, dichloroacetic acid- DCAA, bromochloroacetic acid- BCAA, dibromoacetic acid- DBAA, trichloroacetic acid- TCAA, and bromodichloroacetic acid- BDCAA, and chlorodibromoacetic acid- CDBAA) were purchased in one single mixed solution at a concentration of 2000 ppm (mg/L) suspended in methyl tert-butyl ether (MTBE) from Sigma-Aldrich (St. Louis, MI, USA). Three of the iodinated haloacetic acids were ordered from CanSyn Chem. Corp (Toronto, CA) and IAA was ordered separately through Sigma-Aldrich (Burlington, MA, USA); all were individually dissolved into HPLC-grade MTBE (Sigma-Aldrich). The 4 compounds stock purities are as follows: iodoacetic acid- IAA (>99%), chloroiodoacetic acid- CIAA (90%), bromoiodoacetic acid- BIAA (85%), and diiodoacetic acid- DIAA (90%). All concentrated stocks listed above are combined into one single 100 mg/L mix suspended in methanol (MeOH). This mix is made in methanol to verify that the analyte is water soluble and therefore extracted from the matrix; the extracting solvent used is MTBE. The internal standard (ISTD) used is 1,2-dibromopropane (12DBP, purity 97%, Sigma-Aldrich) in MTBE at a concentration of 30 mg/L.

Liquid-Liquid Extraction:

Each sample begins with an aliquot of 100 mL of ultra-Pure water (MilliQ water, 18 MΩ) in a 125 mL amber bottle (narrow mouth bottle with plastic cap and Poly-Seal® cone liner, Sigma Aldrich). The MiliQ water is then spiked with desired concentration with an air-tight syringe. First the sample is pH adjusted to below 1 with 1 mL of concentrated sulfuric acid (H_2SO_4, Fisher Scientific, Pittsburg, PA, USA), then 5 mL of methyl tert-butyl ether (MTBE), and finally then 30 mg of anhydrous sodium sulfate (Na_2SO_4, Purity ≥99.0%, Sigma Aldrich). The acidification of the sample matrix verifies that the samples are non-dissociated while the addition of salt promotes the analyte to move from the polar phase (water) into the non-polar phase (MTBE). The samples are then placed on a shaker for 15 minutes and allowed to rest for 10 minutes. The MTBE layer is extracted (approx. 5 mL). The addition of 5 mL MTBE, shaking, resting, and extracting is performed 2 more times to ensure all analyte is extracted from the matrix. The approximately 15 mL extract is then run through a sodium sulfate filled pipette to ensure all water is removed. The extracts are then blown down to 200 µL under nitrogen and transferred to an amber GC-vial (AQ™ Brand with AQR™ screw cap, MicroSolve Technology Corp., Eatontown, NJ, USA). The 200 µL extract is spiked with 4 µL of 30 mg/L internal standard (1,2-DBP) and stored in the freezer until derivatization.

Diazomethane Derivatization:

The standard operating procedure used is based on the EPA approved SOP RSB 010.1.[37] All chemicals and diazomethane generator were purchased from Sigma Aldrich. In order to generate diazomethane, 0.367 g Diazald (*N-methyl-N-(p-*

tolysµlfonyl)nitrosamide) and 1 mL of carbitol (2-(2-ehoxyethoxy)ethanol) were added to the inner section of the generator, while 3 mL of MTBE is added to the outer section of the generator. The three generator pieces (shown in figure 2.3) are combined and placed in ice. Once the components of the generator have equilibrated properly in the

Figure 2.3. The diazomethane generator used for diazomethane production and derivatization.

ice, 1.5 mL of 37% KOH was added dropwise (very slowly to avoid pressure build-up). Once the KOH had been fully added, the hood was closed, and the generator was allowed to react for 1 hour. Once completed, the yellow diazomethane collected in the outside section was ready for use.

A ratio of 2:1 is used in the addition of diazomethane for each extract. In this case with a 200 µL extract, 100 µL of diazomethane is added. Once the 100 µL diazomethane is added, the solution reacts for 30 minutes. The reaction is then quenched with 20 mg of silica for 30 minutes. The sample is then transferred into a fresh vial for storage in the freezer for analysis. It is suggested to run samples within one-week post-derivatization.

Steps for Acidic Methanol derivatization:

First, the 200 µL water extract (from LLE process) in an amber GC vial (without an insert) is added with 4 µL of internal standard (30 ppm 1,2-dibromopropane) and 220 µL of 10% conc H2SO4 in MeOH. A 5x2 mm stir-bar is added, and the samples are then placed in a 50 °C water bath for 2 hours. Once the samples have stirred in the water bath for 2 hours, the samples are then cooled to 4 °C in an ice bath for 10 minutes. A 550 µL aliquot of MilliQ is added to each sample in addition to 55 mg of Na_2SO_4, and the samples are shaken by hand for several minutes and set to rest for 5 minutes. The MTBE layer is extracted and transferred into a new vial (approximately 150 µL). Finally, the sample is dried in one final addition of Na_2SO_4 and the sample is placed in its final vial. The samples are then ready for GC-MS analysis.

Steps for GC-MS analysis:

The initial stages of method development were performed using a Thermo Fisher Trace 1310 GC paired with a TSQ Quantum MS/MS (GC-MS/MS) instrument with multiple reaction monitoring (MRM), however due to instrumentation problems, the final replicates shown in data analysis were performed using an Agilent Technologies 6890N GC with a 7683B Series Injector paired with a 5975 inert XL MS (GC-MS). Selected ion monitoring (SIM) was also used for detection at lower levels than with a traditional m/z 33-650 scan; the ability to detect at lower levels allowed us to detect these compounds at realistic levels previously reported in real drinking waters.

2.3 Results and Discussion

The acidic methanol derivatization was adapted from the U.S. EPA Method 552.3

and a method described by Nikolaou *et. al.,*[25] *but* some variations were applied by Jennifer Mueller in 2015[20]. A liquid-liquid extraction was performed 3x instead of a single extraction step in order to increase extraction efficiency; in addition to this, no copper sulfate was used to differentiate between the aqueous and organic layer (blue color) because it can lead to degradation of brominated trihaloacetic acids (TBAA,

Table 2.1. Gas chromatography and ionization conditions for the GC-MS full scan and SIM experiments.

Instrument: Agilent Single Quad		
Gas Chromatography	**GC-MS (m/z 33-650)**	**GC-MS (SIM)**
Column	30 m x 0.25 mm inside diameter (0.25 µm film thickness) RTX-200	30 m x 0.25 mm inside diameter (0.25 µm film thickness) RTX-200
Injection Mode	Splitless	Splitless
Carrier Gas	Helium	Helium
Flow Rate	1.2 ml/min (constant)	1.2 ml/min (constant)
Temperature Program	Temp 35 °C, hold 2 minutes Ramp 9 °C/min to 150 °C Ramp 20°C/min to 280 °C, hold 3 minutes	Temp 35 °C, hold 2 minutes Ramp 9 °C/min to 150 °C Ramp 20°C/min to 280 °C, hold 3 minutes
Injector Temperature	250 °C	250 °C
Sample Volume	1 µl	1 µl
Syringe Cleaning	2x MeOH, 2x Acetone	2x MeOH, 2x Acetone
EI Source		
Filament Emission Current	1.0 mA	1.3 mA
Electron Energy	- 70 eV	- 70 eV
Ion Source Temp	200 °C	200 °C
Solvent Delay	4 minutes	4 minutes

BDCAA, CDBAA). [20] One final step is the addition of the sodium sulfate drying step in order to verify the dryness of the extract when injecting onto the GC-column; this is present in the EPA method 552.3.

In addition to these changes, the addition of a newly developed SIM method for the analytes has proven to show better detection limits for many of the haloacids than

previously reported (Figure 2.3). This new SIM method lowered LOQs for the following compounds: IAA, DBAA, CAA, BAA, DCAA, TCAA, and BCAA. In addition to this, the comparison of acidic methanol derivatized analytes to diazomethane derivatized analytes showed that acidic methanol derivatization is more efficient with some of the brominated haloacids. The following haloacids had comparably better LOQs when derivatized with acidic methanol: DBCAA, BIAA, while DBAA and BAA also had better detection limits than previously reported, they also had the same LOQs with diazomethane derivatization, therefore this is likely attributed to the SIM method. The brominated trihaloacetic acids are generally difficult to consistently quantify at low levels, however, the acidic methanol derivatization promoted a better detection for DBCAA. In addition to the brominated DBPs, CIAA also had a better extraction efficiency and therefore a better LOQ than the diazomethane derivatized replicates. Although acidic methanol derivatization did not improve all LOQ values, it did have significantly better % RSD than its diazomethane derivatized counterpart (Table 2.3), making it possibly more reliable.

2.4. Conclusions

The analysis of haloacetic acids and iodoacetic acids is extremely important due to possible human health effects. Since only 5 of the 9 haloacetic acids are regulated and none of the iodoacetic acids are regulated, the need for drinking water analysis of these compounds continues to grow. Iodoacetic acids are toxic—particularly IAA, which has been shown to cause developmental abnormalities in mouse embryos.[21] To analyze haloacetic acids and iodoacetic acids by GC-MS, derivatization must occur to increase

volatility and decrease polarity for detection with GC. Development of a reliable and practical derivatization method is imperative for monitoring of these compounds.

When comparing the two well-known derivatization methods (acidic methanol and diazomethane), there are many parameters and components to consider. First and foremost, this derivatization method is extremely dangerous because the generation of diazomethane is required; diazomethane is carcinogenic and explosive. In contrast, acidic methanol derivatization requires many more steps than diazomethane derivatization and ultimately introduces much more error. The experiments performed to directly compare the efficiencies of these two methods are described in section 2.2 and 2.3.

Ultimately, the two methods prove to be efficient and provide appropriate detection limits. However, there are 2 compounds (CIAA and DBCAA) that were derivatized more efficiently with acidic methanol than with diazomethane and effectively gave better detection limits. The main source of better detection limits in this set of experiments is attributed to an optimized selected ion monitoring (SIM)-MS method. Of all 13 compounds, 9 of them gave better detection limits than previously reported with a single quadrupole-MS instrument including CAA, BAA, DCAA, TCAA, BCAA, DBCAA, DBAA, IAA, and CIAA.

To summarize this project, the acidic methanol derivatization is almost equal in comparison to the diazomethane derivatization. To improve the acidic methanol derivatization, a double or triple liquid-liquid extraction after the derivatization could show improvement of extraction and derivatization efficiency, as an addition 2

extractions has shown this in full scale water liquid-liquid extractions.[20] Ultimately, the two derivatization methods are comparable, and acidic methanol proves to be a less dangerous and less toxic way to analyze a large fraction of haloacids.

Table 2.2. The SIM method used.

Compound	*Quant Ion (m/z)*	*Qual ion (m/z)*
12DBP (ISTD)	123	121
CAA	77	108
BAA	152	154
DCAA	83	85
IAA	73	200
TCAA	119	117
BCAA	127	129
DBAA	175	173
CIAA	175	234
DIAA	199	326
DBCAA	209	207
BIAA	151	278
TBAA	253	251

Table 2.3. The LOQ values established with new SIM method vs. previously reported values for IAAs and HAAs.

	Previously reported values for LOQ		New SIM Method on Single Quadrupole		n=7	n-7
	Single Quadrupole (SIM)	Triple Quadrupole (MRM)	*Diazomethane Derivatized*	*Acidic MeOH Derivatized*		
Compound	LOQ (ppt)	LOQ (ppt)	LOQ (ppt)	LOQ (ppt)	**Diazo %RSD for LOQ**	**Acidic MeOH %RSD for LOQ**
CAA	50	100	25	25	12.2	7.1
BAA	50	100	25	25	8.4	1.4
DCAA	50	100	25	25	9.8	8.3
TCAA	50	100	25	100	4.0	-
BCAA	100	100	25	25	5.6	3.9
DBCAA	250	x	250	50	-	1.1
BDCAA	50	x	500	500	-	-
DBAA	50	100	25	25	6.8	2.9
TBAA	x	x	x	x	-	-
IAA	50	25	25	25	15.0	2.5
CIAA	125	25	250	50	-	1.4
BIAA	125	50	500	250	-	-
DIAA	50	50	x	x	-	-

*Table 1*Note: the "x" indicates this compound was unable to be quantified reproducibly (no LOQ value listed as of now).*

CHAPTER 3: VACUUM ASSISTED SORBENT EXTRACTION (VASE) ANALYSIS OF PRIORITY AND EMERGING DISINFECTION BY-PRODUCTS

3.1 Introduction and Background

Extraction from complex matrices has been an issue to overcome by chemists and engineers for many decades; particularly the extraction of volatiles from these matrices has proven to be difficult. Furthermore, when analytes exist at traces levels there is an even more complex issue at hand. Because of this problem posed by scientists all over, headspace methods have become extremely popular. Throughout the 21st century, the analysis of disinfection by-products (DBPs) has changed dramatically. Some of the progress in this research field has centralized on developing methods for extracting VOCs/SVOCs from their matrices before analysis by either GC-MS or LC-MS. To this point, these methods have traditionally included liquid-liquid extraction (LLE), solid phase extraction (SPE), and XAD resin capture. More recently, methods involving extraction using the headspace of sample vial, such as Solid Phase Micro-Extraction (SPME), have gathered attention because of their propensity to avoid matrix effects.

Headspace extraction is when the analyte of interest is captured from the gas phase above the sample and adsorbed onto a solid phase.[40] Though using SPME might mitigate matrix effects, the reproducibility of a micro-extraction suffers in comparison to a larger sample volume extraction, such as LLE. An interesting alternative headspace

extraction method is vacuum assisted sorbent extraction (VASE). This novel method has the potential for many advancements in extraction of volatiles in complex matrices such as wastewater, biological samples, urine, and more. VASE is a variation of the known headspace methods already evaluated in the analysis of DBPs, in which the addition of a vacuum gives better extraction efficiencies, thereby increasing the reproducibility of the method.[41,42] This means that a 1 mL sample of drinking water can give the same if not exceed the detection limits of a 100 mL liquid-liquid extracted sample (Figure 3.6).

To determine if this method is potentially viable for the analysis of DBPs, much development and optimization is required. Each class of DBPs must be analyzed and optimized individually, as each of nearly 60 compounds that are analyzed with respond with some variability. In addition to the "full-list" (Table 1.1) that is commonly analyzed via LLE, there are three classes of priority DBPs that require some sort of derivatization for GC-MS analysis. These classes include the haloacetic acids (HAAs), the iodoacetic acids (IAAs), and the haloacetaldehydes (HALs).

Figure 3.1. Example of SPME vs. VASE- comparison of microextraction versus an exhaustive extraction VS LLE.

VASE utilizes a compound's inherent volatility in addition to the application of 30 mm Hg (torr) vacuum to drive analytes of interest into the headspace. Once in the

headspace, the analytes sorb to the solid phase, and are then ready for thermal desorption for subsequent analysis by GC-MS. The fundamental idea behind a compound's tendency to enter the gaseous phase from the liquid phase (water sample matrix) is found in Henry's Law constant (Eq. 1) where the units for a Henry's Law Constant (or k value) are often reported as atm-m^3/mole. Henry's Law relates a compounds concentration (mol/L) to the partial pressure (mm Hg) of that compound. The higher a compound's k value, the more likely it is to volatize from the liquid phase into the gaseous phase. The lower an analyte's k value, the more persistent the compound is in water, and the lower the likelihood that the analyte will volatilize and sorb to the HS-pen solid phase.

$C=k*P$ Eq. 1

Several parameters will be discussed in the following sections including pH, ionic strength (salt addition), extraction parameters (agitation and temperature), and desorption parameters (preheat and thermal desorption temperature) as well as a few examples of optimization. The focus of this project is unregulated disinfection by-products, although the regulated trihalomethanes (THM4) are to be included in the method in the future.

3.2 Materials and Methods

The VASE system by Entech Instruments[42,43] was paired with a Thermo Scientific Trace 1310 gas chromatograph-TSQ 9000 triple quadrupole mass spectrometer for GC-MS and GC-MS/MS analysis. Although the Richardson lab has this system, some of the data shown in the following experiments were derived from data acquired on a Thermo

Scientific Trace 1310-ISQ 7000 single quadrupole mass spectrometer located at Entech Instruments in Simi Valley, California, USA.

Sample preparation, extraction of analytes, and preconcentration steps with VASE can be compounded into just a few steps (Figure 3.2 and Figure 3.3). The sample was transferred into the vial and the Headspace Sorbent Pen was loaded into the sealed lid. Once sample was prepared, a vacuum is applied using a Vacuubrand vacuum pump MZ-2-NT. Vacuum was applied for approximately 30 seconds to achieve a vacuum of 28-30 mm Hg inside the sample vial. Then, a controlled headspace extraction occurs using the Entech Instruments 5600 Sorbent Pen Extraction System (SPES) for 30 minutes-24 hours depending on target analyte volatility. For most experiments an agitation and extraction was applied for 16 hours.

Extraction was performed at the desired temperature up to 70 °C and agitation speeds up to 300 RPM. Step 3 was a water control step—samples are loaded onto a -40°C cold tray in order to drive the water back into the vial and off of the solid phase; this was done to avoid loading water onto the analytical column. The HS-sorbent pens were then placed into their isolation sleeves until they were ready for analysis.

Thermal desorption took place in the VASE desorption unit (Figure 3.3) and extracted analytes were analyzed by GC-MS. Once the sample was desorbed, the sorbent pens were ready for re-use. If needed, the sorbent pens were be conditioned with an Entech Instruments 3801 Sorbent Pen Thermal Conditioner (SPTC). Conditioning was done at any time and was sometimes be necessary to avoid carry-over from a dirty sample matrix (such as urine).

First and foremost, the headspace pen (HSP) contains a septum-less seal (Figure3.2), and several o-ring seals to assist in sealing vacuum. In addition to this, the sorbent bed is within the headspace pen. The sorbent can be variations of Tenax, Tenax and Carboxen, etc. The sorbent used in this method is Tenax 35/60; each pen has approximately the same amount of sorbent installed from pen to pen.

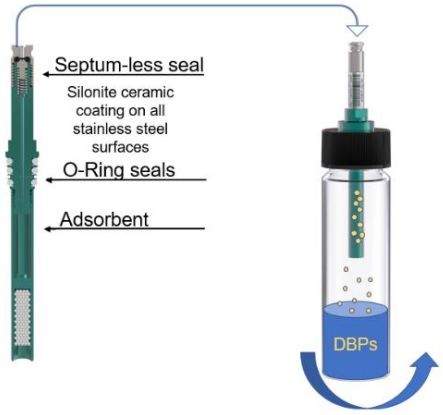


Figure 3.2. The effect that vacuum, agitation, and heating has on samples extracted with VASE.

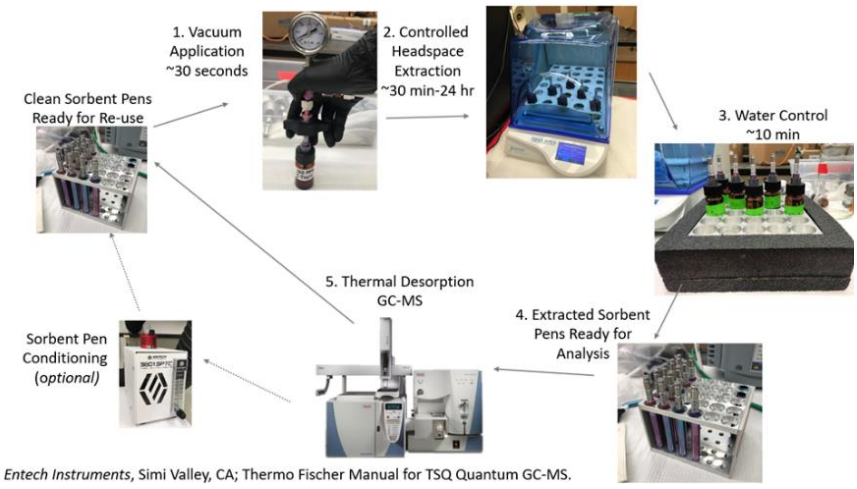


Figure 3.3. A brief overview of the VASE method including sample preparation and GC-MS analysis.

VASE: How Does it Work?

Extraction works by first transferring 1 mL of sample containing analyte into the HSP vial. The vial was then sealed with a series of plastic and silonite pieces –the metal sealing silonite pen holder, the top plastic black lid to screw the silonite pen holder to the vial, and finally the pen acted as the final sealing piece. The vial was then placed under vacuum, promoting the movement of VOCs and SVOCs to the headspace (and ultimately on the sorbent bed within the HSP). The sample vial was then moved to the 5600 SPES for agitation and heating. These extractions take place between 2 hours and 24 hours, agitation is between 200-250 RPM, and heating takes place between 30 °C (86 F) and 70 °C (158 F). General parameters used in the following experiments were as follows: agitation at 225 RPM for approximately 16 hours. Samples were extracted at 30 °C, 50 °C, and 70 °C to test for the best extraction temperature.

Because the analysis of so many diverse compounds was being optimized, the analytes were broken down into their DBP class. These classes are based on the core properties of the analytes such as volatility, general mass, general structure. For example, the haloaldehydes are likely to respond similarly to the change of parameters based on the molecular weight, polarity, and boiling point, so they were placed in the same class.

Parameters to Optimize:

Extraction Parameters:

Agitation Speed, Extraction Time, and Extraction Temperature:

Each class of DBPs will react differently to extraction agitation and temperatures due to the molecular weight, volatility, and Henry's law constant of each compound. Because of this, the compounds are broken into their respective classes and extracted at 30 °C, 50 °C, and 70 °C with the same agitation speed at each temperature. After this, the compounds are tested with the optimal temperature at various agitation speeds. In addition to optimizing for the agitation speed and temperature, the compounds are also tested for the optimal extraction time. In general, agitations may run from 200-250 RPM, and may take place anywhere from 30 minutes to 24 hours.

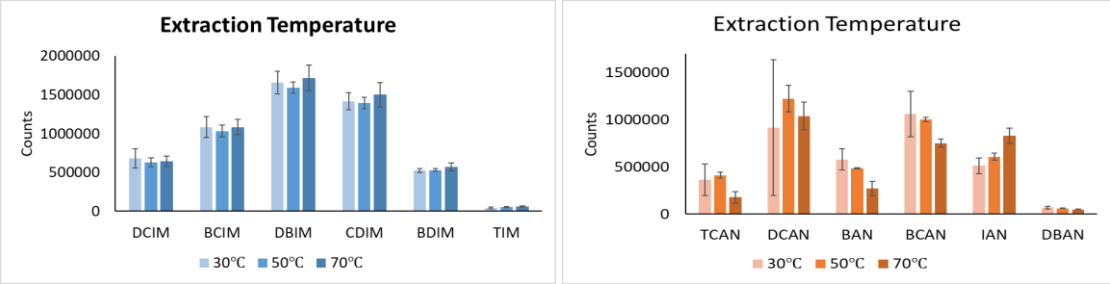


Figure 3.4. Two classes of DBPs—the I-THMs (blue) and the HANs (orange); 3 different extraction temperatures explored: 30 °C, 50 °C, and 70 °C.

pH:

The pH of a mixture is one of the most important steps in extraction of target analytes from a particular matrix. The optimal pH for an analyte is dependent on the pKa of that VOC/SVOC. At the target analyte's pKa, it exists in an equal mixture of its protonated and deprotonated form (50:50). Because of this, during extraction the

matrix was first pH adjusted to below 1. The pH of below 1 was chosen because the pKa range of target analytes are all above 1 except for the haloacids (HAAs). This pH adjustment is so important for promotion of VOCs and SVOCs into the headspace because they must be protonated in order to volatilize. In Figure 3.5., triiodophenol (TIPh) has a pKa value of close to 25, while triiodomethane (TIM) has a pKa value of 6.46, and because of their pKa values, the pH adjustments altered the value of 6.46, and because of their pKa values, the pH adjustments altered the extraction efficiency of both.

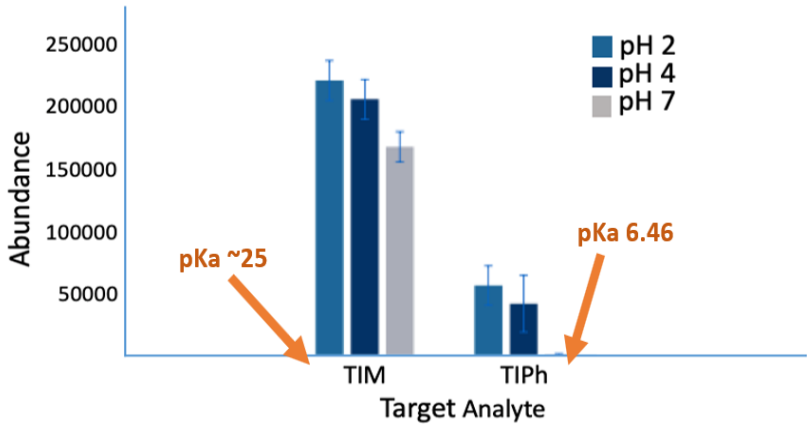


Figure 3.5. An example of the extraction efficiency changes of 2 analytes with very different pKa values. (Simi Valley, CA)

Salt Addition:

Ionic strength is a large component of extraction techniques. The addition of salt to a sample matrix is important because it increases the extraction efficiency by promoting the movement of VOCs and SVOCs to move from the polar (water) phase to the nonpolar phase (solvent of choice, often MTBE) by increasing ionic strength. For example, the traditional LLE used in the Richardson lab follows a sodium sulfate addition to increase ionic strength and promote the movement of the volatile and semi-volatile

DBPs from the polar phase (water) to the MTBE phase (nonpolar). In the case of headspace extraction, the use of salt allows for the movement of the same volatile and semi-volatile DBPs from the polar water phase into the headspace and ultimately adsorbing to the Tenax 35/60 phase.

Water Removal Step:

Because of the propensity for water to destroy hydrophobic columns, allowing it to enter a GC-MS system is typically avoided at all costs. Because of this and the nature of VASE extraction, it was extremely important to verify that water was not left on the sorbent bed of the headspace pen. To make sure water is not on the pen after extraction, a water removal step took place (Figure 3.3).

Desorption Parameters:

Preheat Duration and Preheat Temperature

The preheat of these compounds while on the sorbent phase is extremely important because it allows the analyte to be warmed to an appropriate temperature so that it is transferred onto the column quickly (leading to better peak shape and signal-to-noise (S/N)).

Once the best preheat temperature was optimized, the preheat length of time was the second important factor of desorption development. Figure 3.6 shows a class of DBPs (the I-THMs) being preheated at 250 °C for 3 different time frames (0.5 min, 1 min, and 2 mins). The response of the I-THMs and HAMs is optimized with a short and very hot preheat. Refer to Figure 3.7 for an example of 2 compounds signal-to-noise (S/N) changes with 2 different preheat durations at the same temperature. For both

compounds, the peaks on the left (best S/N) are indicative of a 0.5 minute preheat at 250 °C, while the right (worse S/N) is representative of a 1 minute preheat at the same temperature. DCIM is dichloroiodomethane and CAM is chloroacetamide. CAM often has poor S/N and spreads out on the column, often causing chromatography problems and lower detection limits in DBP analysis. With VASE headspace analysis, the act of preheating the compounds on the sorbent bed allows for a much cleaner transfer into the GC-column, resulting in better S/N.

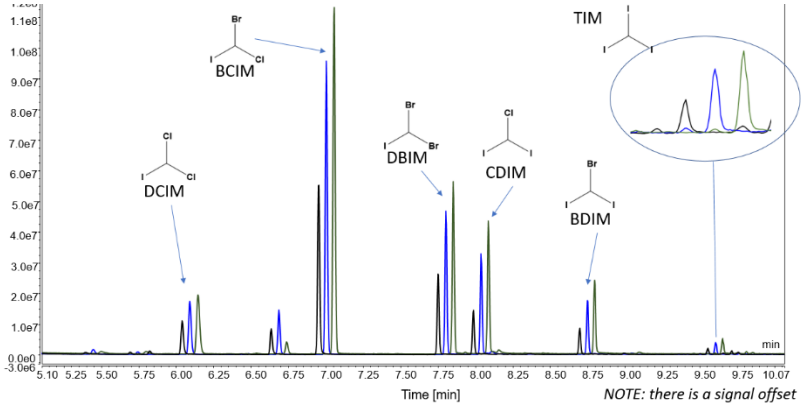


Figure 3.6. Example of a DBP class (the I-THMs) being preheated at 250 °C for 3 different time frames (0.5 min, 1 min, and 2 min).

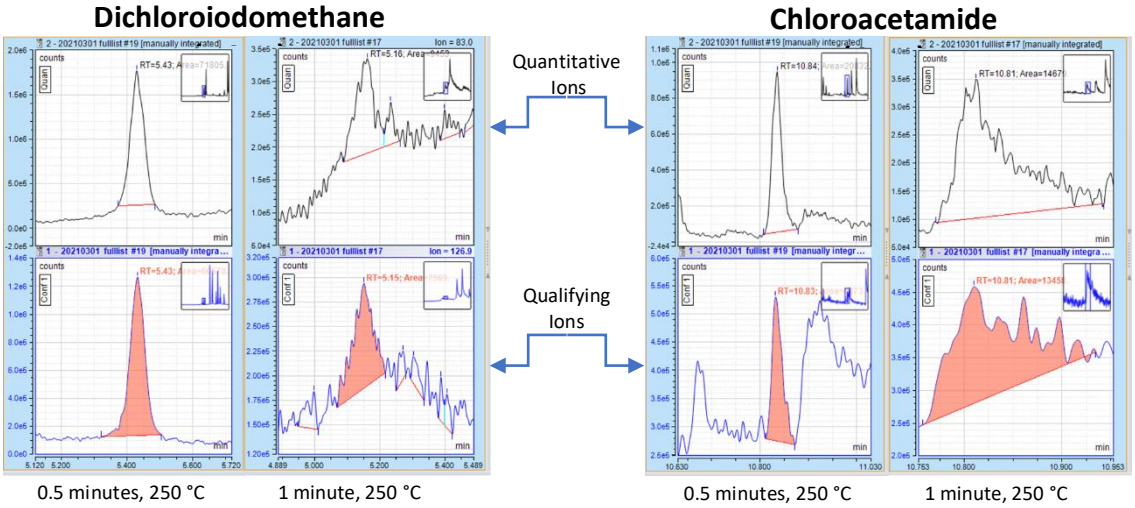


Figure 3.7 An example of how important the preheat temperature is in terms of signal-to-noise and analysis of compounds on a GC-column.

Desorption Temperature and Desorption Duration

The act of thermal desorption of VOCs and SVOCs has been around for a long time, however, recently with the addition of a sorbent phase and preheat of the sorbent has potential to allow for better detection limits for several reasons. Along with the importance of preheat (as described above), the duration and temperature at which a compound is desorbed is one of the biggest determining factors of GC-analysis and S/N on the GC-column.

To further explain, one must look at the established methods in the Richardson lab. In the approximately 70 DBPs analyzed in the Richardson lab, there is a small list of thermally labile compounds that require a multi-mode inlet (MMI) to get appropriate detection limits. A few of these compounds fall in the halonitromethane (HNM) class of DBPs; the trihalonitromethanes tend to degrade much more easily at high temperatures. For analysis of these thermally degrading compounds, the MMI is programmed to begin at 170 °C and heat up to the usual 250 °C. For analysis of all other compounds, an inlet at 250 °C is acceptable, however, the thermally labile compounds (we call this our “problem”-list) require a much lower inlet temperature. Because of this, the VASE desorption unit is optimal for analysis of these compounds in addition to the ~60 others. The VASE desorption unit allows for a multi-temperature preheat and desorption, similar to an MMI. The temperature requirements for the target volatiles are met with ease using the VASE desorption unit.

3.3 Results & Discussion

The initial stages of method development began with 3 of the 7 total DBP classes analyzed in the Richardson lab. These classes are the haloacetonitriles, the iodo-trihalomethanes, and the haloacetaldehydes. These classes were chosen because they cover a large range of volatility and molecular weights. In addition to this, the haloacetaldehydes require an in-vial derivatization with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (referred to as PFBHA).[44] After the initial method development of these classes, the remaining 4 classes have been partially explored. An appropriate preheat and desorption has been established for all compounds, including the "problem"-list of thermally labile compounds—this will be further explained in the following section.

Methods Developed for the I-THMs and HANs

As stated in section 3.2, there are many parameters that must be explored for these versatile SVOCs and VOCs. First, the classes of compounds are directly spiked onto the HSPs for initial desorption and preheat analysis. The initial trials include the following preheat temperatures: 70 °C (the desorber idle temperature), 100 °C, 150 °C, and 250 °C. Preheat durations are tested at 0.5 minutes, 1 minute, and 2 minutes. Once preheat is established for optimal peak shape and S/N. the desorption parameters are explored at various temperatures and durations. These temperatures vary entirely on the target analytes and their volatility and thermal sensitivity. Once the thermal desorption parameters and GC method has been optimized, the extractions can begin. Extractions of these classes took place at 30 °C, 50 °C, and 70 °C (keeping agitation

speed consistent). Recovery of the I-THMs and HANs extracted at these temperatures are shown in figure 3.8, and recoveries are comparable and often better than the recovery given by the established LLE method.

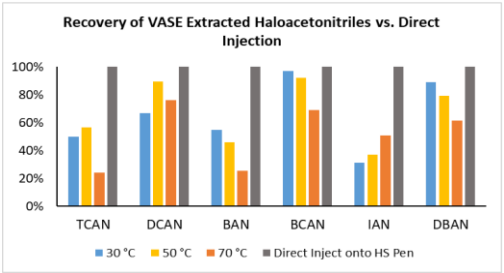
Ultimately, the optimal preheat setting for both classes is a short and hot preheat for 0.5 minutes at 250 °C. The desorption of these compounds varies a little bit; the class of I-THMs does best with a 1 step desorption at 250 °C, while the HANs do best with a 2-step desorption (D1 and D2 are desorption 1 and desorption 2) of D1 for 3 minutes at 300 °C and D2 for the remaining time at 250 °C. A full table of optimized parameters for these classes is shown in table 3.1.

Table 3.1 The optimized desorption methods for the Iodo-Trihalomethanes and Haloacetonitriles.

Haloacetonitriles Method		**Iodo-Trihalomethanes Method**	
Parameter	***Duration and Temperature***	***Parameter***	***Duration and Temperature***
pH	< 1	pH	< 1
Agitation	16 hours, 50 °C at 225 rpm	Agitation	16 hours, 70 °C at 225 rpm
Preheat	0.5 minutes at 250 °C	Preheat	0.5 minutes at 250 °C
Desorption	3 minutes at 300 °C	Desorption	10 minutes at 250 °C
	7 minutes at 250 °C		no D2
Bake Out	3 minutes at 250 °C	Bake Out	3 minutes at 250 °C
Post Bake	7 minutes at 70 °C	Post Bake	7 minutes at 70 °C

Analyte	VASE Recovery	LLE Recovery
TCAN	57%	20%
DCAN	89%	48%
BAN	46%	39%
BCAN	92%	56%
IAN	37%	45%
DBAN	79%	63%

Analyte	VASE Recovery	LLE Recovery
DCIM	60%	59%
BCIM	67%	52%
DBIM	77%	57%
CDIM	78%	55%
BDIM	89%	50%
TIM	135%	39%



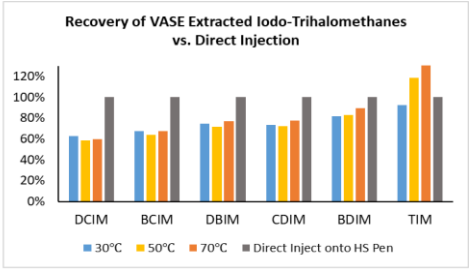


Figure 3.8. The recovery of the I-THMs and HANs extracted with VASE versus the traditional LLE performed in the Richardson lab.

Method Developed for HALs and In-Vial Derivatization

The halo-acetaldehydes (HALs) require derivatization by PFBHA, which is an in matrix (water) derivatization, the recoveries of these compounds have not been established. Further explanation is that the compounds must be derivatized in water prior to extraction, so we were unable to spike them directly onto the pen for recovery tests. Because of this, all optimizations must take place post-extraction. In figure 3.7 the HALs are tested for optimal extraction temperature and linearity. The preheat and desorption parameters optimal for this class were the same as the HANs (Figure 3.8). Further optimization of the haloaldehydes included testing various amounts of PFBHA addition, development of a SIM method (figure 3.10), and in the future development of an SRM method for even lower detection limits. Detection limits are shown in table 3.2.

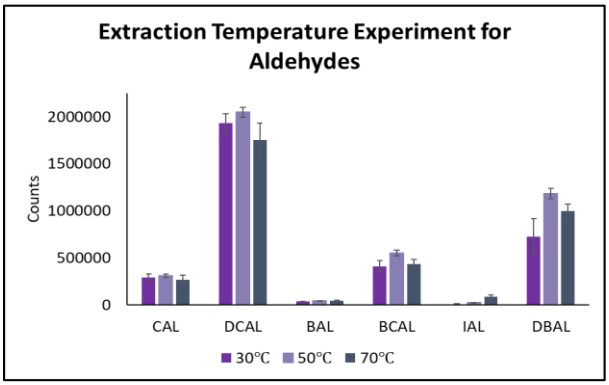


Figure 3.9. Extraction temperature optimization for the haloacetaldehydes; variations at 30 °C, 50 °C, and 70 °C.

Table 3.2 describes the preliminary detection limits for the VASE extracted halo-acetaldehydes analyzed by GC-MS.

Compound	Detection Limit (ppt)
CAL	50
BCAL	50
DCAL	50
BAL	50
IAL	100
DBAL	1000

The final step in optimization of the HALs is testing multiple amounts of PFBHA for derivatization efficiency of spiked matrices (MilliQ water was tested). Overall, the addition of just 1 mg/mL of PFBHA is required for full analysis of all HAL DBPs tested at 100 µg/L (ppb).

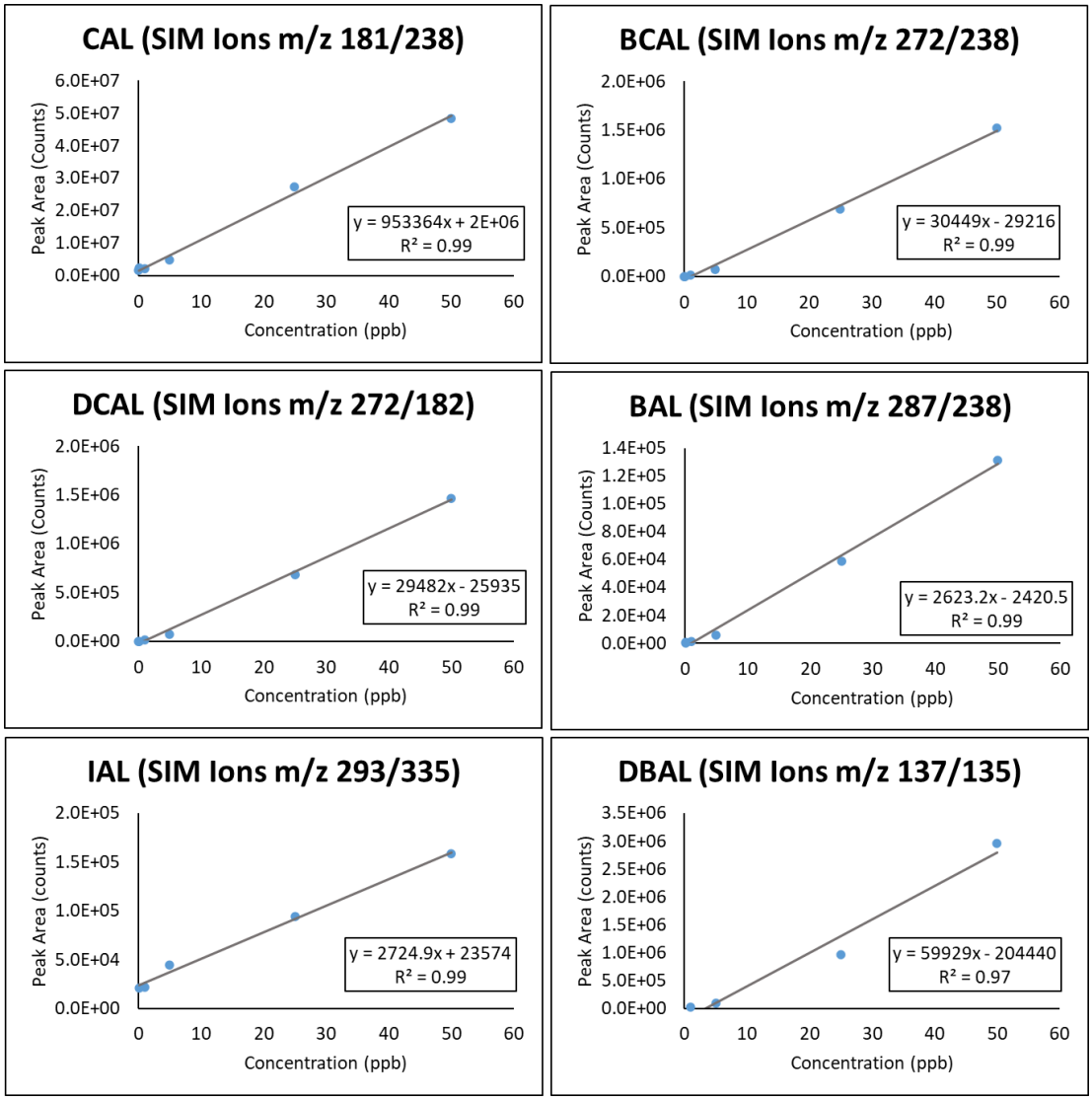


Figure 3.10. The HALs selected ion monitoring (SIM) method developed for LOD and LOQ development.

Development of the remaining DBPs

Once established methods had been developed for the I-THMs, HANs, and HALs the next focus was on the thermally sensitive group of compounds (p-list or "problem"-list). Focusing on the most thermally sensitive DBPs opens the opportunity to include them within one method for all 48 target DBPs (the 6 p-list, 4 regulated THMs, 9 HKs, 6 I-THMs, 12 HAMs, 4 HNMs, and 7 HANs). Although steps were taken to optimize for the HANs and I-THMs separately, the goal is to make one singular method for analysis of all 48.

As mentioned before, preheat is extremely important, especially for thermally labile analytes. Since the P-list is thermally sensitive, multiple preheat temperatures and even 2-step preheats were explored. Refer to figure 3.9 for results of these experiments. A preheat of 0.5 minutes at 170 °C is optimal for these compounds. In 2004, in a study by Plewa *et al.,* involving the thermal sensitivity of the trihalonitromethanes, 170 °C was found to be optimal for the trihalonitromethanes (such as DBCNM and TBAN), therefore this temperature was chosen.[45] Once preheat was determined, the desorption parameters were tested at various temperatures; the optimal method determined for these compounds can be found in table 3.4. This method was then tested for the remaining 42 compounds.

As of now, one singular method has been developed for the analysis of 44 total DBPs usually analyzed in the Richardson Lab by GC-MS. The 44 excludes the haloacetaldehydes (HALs), the haloacetic acids (HAA9) and the iodoacetic acids (IAAs) due to their derivatization steps. The method also did not test for the 4 regulated THMs

but will eventually include them. Initial method development for one singular method to include all 44 compounds is performed with an n=3 at 100 µg/L direct injections onto the HSP (table 3.3).

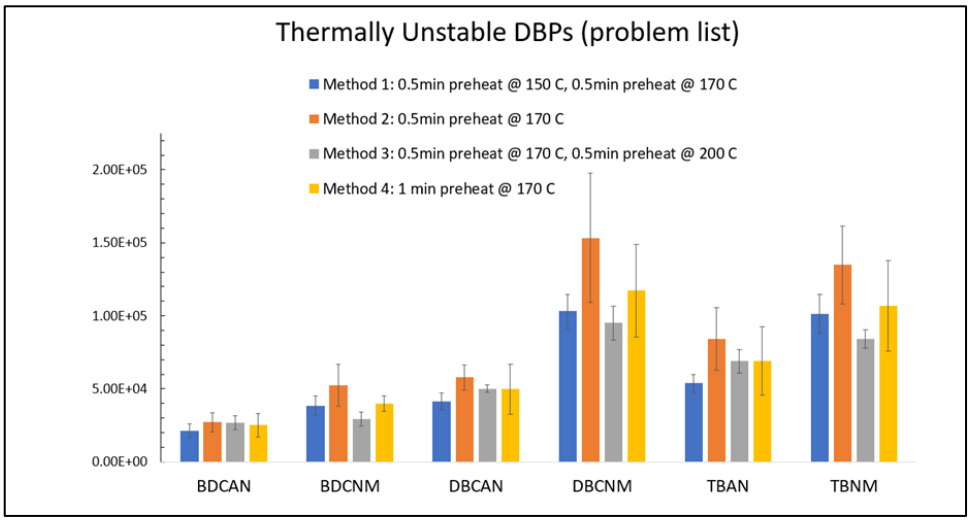


Figure 3.11. The results of preheat experiments on the most thermally labile target analytes.

Table 3.3. The 44 DBPs successfully analyzed by one method utilizing GC-MS paired with VASE thermal desorption.

Class	Compound	RT (min)	% RSD	MS Scan Type
P-list	BDCAN	5.48	2.72	SIM
P-list	BDCNM	7.35	5.92	SIM
P-list	DBCAN	7.41	2.81	SIM
P-list	DBCNM	9.17	6.18	SIM
P-list	TBAN	9.39	3.52	SIM
P-list	TBNM	10.90	6.49	SIM
HK	CP	5.81	2.64	Full Scan
HK	111TCP	7.51	23.27	Full Scan
HK	11DBP	8.63	9.28	Full Scan
HK	1B11DCP	9.13	17.59	Full Scan
HK	13DCP	9.31	38.48	Full Scan
HK	113TCP	10.20	1.40	Full Scan
HK	11DCP	10.23	1.42	Full Scan
HK	1133TeCP	11.03	4.10	Full Scan
HK	1133TeBP	16.17	2.01	Full Scan
I-THM	BCIM	6.76	6.45	Full Scan

I-THM	DCIM	6.76	7.50	Full Scan
I-THM	DBIM	8.29	4.49	Full Scan
I-THM	CDIM	8.68	5.13	Full Scan
I-THM	BDIM	10.21	0.83	Full Scan
I-THM	TIM	11.99	5.18	Full Scan
HAM	CAM	10.57	8.50	Full Scan
HAM	DCAM	12.27	12.75	Full Scan
HAM	BAM	12.44	0.78	Full Scan
HAM	BCAM	13.67	9.74	Full Scan
HAM	TCAM	14.00	10.39	Full Scan
HAM	IAM	14.53	44.09	Full Scan
HAM	DBAM	14.86	6.77	Full Scan
HAM	CIAM	15.40	29.69	Full Scan
HAM	DBCAM	16.53	10.83	Full Scan
HAM	BIAM	16.56	14.76	Full Scan
HAM	DIAM	18.20	14.12	Full Scan
HAM	BDCAM	23.57	4.93	Full Scan
HNM	TCNM	5.41	2.84	Full Scan
HNM	DCNM	5.75	2.86	Full Scan
HNM	BCNM	7.35	10.46	Full Scan
HNM	DBNM	8.88	6.79	Full Scan
HAN	TCAN	4.08	2.88	Full Scan
HAN	DCAN	5.21	4.98	Full Scan
HAN	CAN	5.34	3.94	Full Scan
HAN	BCAN	6.86	3.55	Full Scan
HAN	BAN	6.97	3.62	Full Scan
HAN	DBAN	8.61	7.82	Full Scan
HAN	IAN	9.21	7.87	Full Scan

Table 3.4. An overview of the method optimized to analyze 44 target analytes.

VASE Method (n=3)	
Split Ratio	10:1
Column	RTX-200, 30 m, 0.25 mm x 0.25 µm
GC time	43 minutes
Idle Temp	70 °C
Preheat	0.5 minutes at 170 °C
Desorption	5 minutes at 170 °C
	10 minutes at 250 °C
Bake out	6.3 minutes at 250 °C
	30 minutes at 280 °C

Extracting DBPs from Complex Matrices

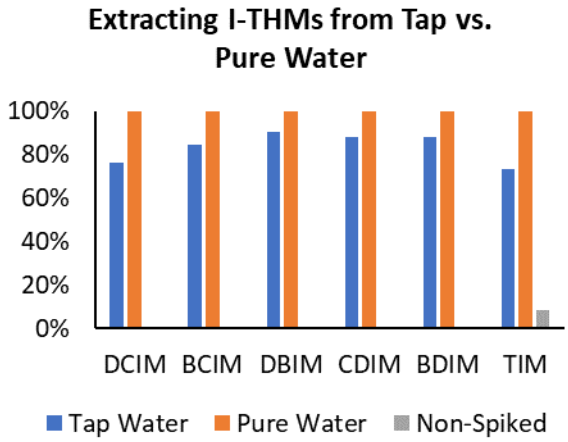
Many complex matrices such as wastewater, urine, blood, and even tea or coffee have proven to be difficult to extract VOCs and SVOCs from. Because of this issue, the Richardson lab proposed the use of headspace analysis with VASE to avoid matrix complications. In order to explore if vacuum assisted extraction (VASE) can truly avoid matrix affects in GC-MS analysis, multiple experiments were performed.

Experiment 1: Extraction of Iodo-THMs from Simi Valley, CA Tap Water (ozonated)

To verify that the proposed method for I-THMs works under more complex circumstances, extractions of the analytes were performed at 25 ppb spiked into Simi Valley, CA tap water. These spiked extracts were also compared against non-spiked tap water and extracted I-THM values from pure water. Future studies will replicate this with the full 48 target DBPs. Finally, a comparison of the I-THM levels detected from a previous I-THM LLE was performed; the comparison is not to be mistaken for "direct" because DBP levels vary throughout the year due to seasons and changes in NOM. The comparison in Table 3.5 is to show that the VASE method is comparable to LLE in terms of DBP extraction. Table 3.5 is a comparison of LLE analyzed tap water from the same tap water as VASE analyzed non-spiked tap water from the same plant.

Experiment 2: Extraction of Iodo-THMs from urine

Urine has shown to be an extremely difficult matrix to extract from because of its high concentration of salt and other biological components. In addition to this, Urine has shown promise for indicating human DBP exposure.[19] Because of this, an experiment with I-THMs spiked into real urine was performed (Figure 3.13.).

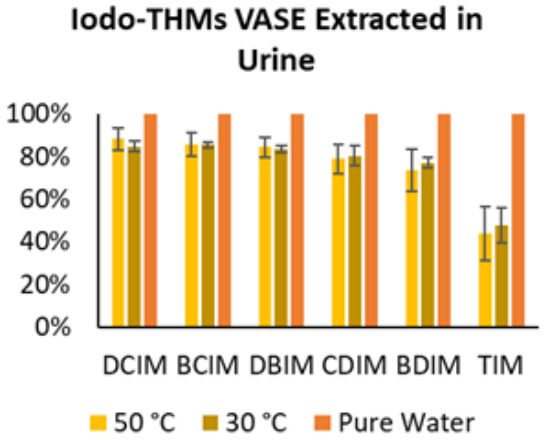


I-THM	Tap Water	Pure Water	Non-Spiked
DCIM	76%	100%	1%
BCIM	84%	100%	0%
DBIM	90%	100%	0%
CDIM	88%	100%	0%
BDIM	88%	100%	0%
TIM	73%	100%	9%

Figure 3.12. Tap water spiked with 25 ppb iodo-THMs and tested against non-spiked tap water and pure water. **Note: "pure water" was set to 100% for comparison; this does not indicate that the extraction efficiency is 100%.*

Table 3.5. Comparison of LLE to VASE analyzed tap water from the same DWTP.

I-THM	VASE Conc. (µg/L)	LLE Conc. (µg/L)
DCIM	1.2	<0.1
BCIM	0.6	0.1
DBIM	ND	0.2
CDIM	<0.5	ND
BDIM	<0.5	<0.1
TIM	1.5	ND



I-THM	50 °C	30 °C	Pure Water
DCIM	88%	85%	100%
BCIM	85%	85%	100%
DBIM	84%	83%	100%
CDIM	79%	80%	100%
BDIM	74%	77%	100%
TIM	44%	48%	100%

Figure 3.13. The extraction of Iodo-THMs from real urine at 30 °C and 50 °C in comparison to the same analytes extracted from pure water. **Note: "pure water" was set to 100% for comparison; this does not indicate that the extraction efficiency is 100%.*

3.4 Conclusions & Future Studies

Overall, VASE analysis shows promise of better detection limits for trace levels of DBPs than currently reported now. With the addition of MS Scan modes SIM and SRM, these LOD and LOQ levels are likely to be even closer to 1 ppt or lower. Further optimization for the 48 target analytes includes development of even better SIM and SRM methods (currently being developed in the Richardson Lab) for the remaining 48 analytes. SIM methods have been developed on an Agilent single quadrupole mass spectrometer and are used daily in the Richardson lab for all 61 DBPs analyzed by GC-MS, however more optimization is possible with the most recent instrument addition to the lab: the Thermo TSQ 9000 MS/MS paired with an AS-1310 and Trace 1310 GC. This MS/MS technology paired with the exhaustive vacuum assisted sorbent extraction and thermal desorption will lower the detection limits at which we see our target analytes and prove to allow us to extract from even the most complex matrices.

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