Optimal Extraction of Nitrogen Containing Disinfection Byproducts From Drinking Water Through Solid Phase Microextraction

an Honors Project submitted by

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ABSTRACT

The aim of this research is to optimize an extraction method to monitor nitrogen containing disinfection byproducts (N-DBPs) in drinking water. Thirty compounds were investigated from three chemical families – haloacetonitriles, halonitromethanes, and haloacetamides– and were extracted using solid phase microextraction (SPME). The method parameters optimized were extraction time, sample temperature, stirring, holding time, and use of an internal standard. The best result was chosen as the optimal setting for use in the final extraction method. This method is intended to be used in conjunction with the EPA to monitor these carcinogenic and genotoxic compounds in drinking water from various municipalities across the United States.

INTRODUCTION

1. Water Treatment

Water is an essential part of all life on Earth and affects our lives in a variety of ways. The United States uses approximately 408 billion gallons per day and of this, 43.3 billion gallons are supplied for public use (Hutson 2004). To be able to use our current water supply, raw water from rivers, lakes, and streams must be disinfected for safe public consumption. Providing safe drinking water is a public health issue and is essential for the modern developed world.

Disinfection is the process that water treatment facilities use to kill bacteria and other microorganisms in raw water. The process of disinfection is essential to having water that is safe for consumption; it can be accomplished using a variety of methods. According to the Environmental Protection Agency (EPA),

> Disinfection is usually a chemical process used in water systems which either chemicals are added to inactivate (or kill) pathogens (i.e., disease causing organisms) found in the source water (i.e., lake, river, reservoir, or ground water aquifer from which water is drawn and treated). Disinfection through inactivation usually involves the use of disinfectants such as chlorine, ozone, and chlorine dioxide, and a combination of chlorine and ammonia (chloramines) may render many of these organisms harmless (Environmental Protection Agency 2008).

The quote illustrates that disinfection is an essential process in providing safe water. The current process of disinfection, however, may form several harmful organic compounds known as Disinfection Byproducts (DBPs).

2. Disinfection Byproducts

DBPs were discovered in 1974 by chemist J.J. Rook and have since been a concern for the EPA. Rook reported the identification of the first DBPs--chloroform and the other trihalomethanes (THMs)—that are formed in chlorinated drinking water (Rook 1974). Over the

past thirty years extensive research has been done on these compounds. In 1976 the National Cancer Institute published the findings of several epidemiological studies that showed that chloroform was related to some forms of cancer (National Cancer Institute 1976). This initial study caused the EPA to determine that in high concentrations these compounds are harmful for human consumption. Due to the harmful nature of these compounds the EPA has developed standards and regulations for the amount of DBPs allowed in processed drinking water. Controlling DBPs is a concern for the EPA, and with the discovery of new DBPs it is becoming an increasingly difficult task.

3. Nitrogen-containing DBPs

Although several DBPs were discovered, a large percentage of organic halides had not been identified in drinking water due to a lack of accounting for total number of halogens known to be present. Thus, scientists assumed that other toxic/carcinogenic compounds were present in the water. Since some halogenated organic compounds are carcinogenic, there was a need to identify those compounds, and more research was done. Scientists still do not have the complete picture of all the DBPs present in water, but as time goes on and technology advances the picture is becoming clearer.

In the last 20 years, improvements in technology and instrumentation have allowed new DBPs to be identified. A new class of DBPs recently identified in chlorinated drinking water is a group of halogenated, nitrogen-containing compounds. The nitrogen-containing DBPs fall into several main classes: halonitromethanes, halonitriles, haloacetamides, and nitrosamines.

Haloacetonitriles (Figure 1a) are organic compounds that consist of an acetate derivative group, known as a cyano (-CN) group, and up to three halogens replacing the hydrogen atoms.

Halonitromethanes (Figure 1b) are a family of DBPs that consist of a nitro group (-NO_2) attached to a single carbon atom with up to three halogens replacing the hydrogen atoms. Haloacetamides (Figure 1c) are a family of DBPs that consist of an amide group (-NH_2) attached to the carboxyl end (-CO-) of a two carbon chain with up to three halogens replacing the hydrogen atoms of the carbon. Another type of N-family of DBPs is nitrosamines (Figure 1d) which even though they were not included in this study, are in fact a class of N-DBPs. Although the later compounds have been indentified in drinking water scientists are unsure of their mechanism of formation.

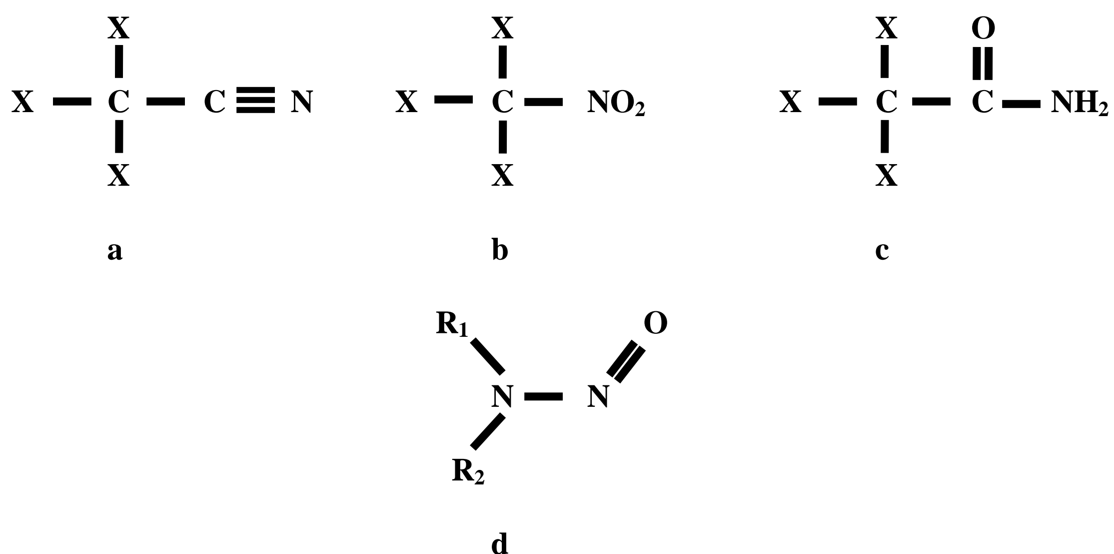

Figure 1: General Structures of Nitrogen Containing Disinfection Byproducts where each X is a hydrogen atom or any halogen. a) Haloacetonitrile b) Halonitromethane c) Haloacetamide d) Nitrosamine

Although the nitrogen-containing DBPs are very similar to the previously discovered DBPs from the 1970's, there are many differences in the two types of DBPs. The nitrogen-containing DBPs, just as earlier discovered DBPs, have been found to be harmful in a variety of ways and are a research priority according to the U.S. E.P.A. (Krasner 2006; Woo 2002) These DBPs have been found to be genotoxic (detrimental to the genetic material of the cell, usually resulting in cancer), and cytotoxic (detrimental to the cell's general structure, usually resulting in

cell lysis). A study published in 2004 concluded, “The halonitromethanes are potent mammalian cell cytotoxins and genotoxins and may pose a hazard to the public health and environment.” (Plewa 2004)

There is more concern with the nitrogen-containing DBPs than earlier DBPs because a recent study concluded, “As a chemical class, the (haloacetonitriles) HANs are more toxic than regulated carbon-based DBPs, such as the haloacetic acids” (Muellner 2007). In theory, the N-DBPs are thought to be more toxic because they are able to attack the DNA more easily than non-nitrogen containing DBPs. The potentially dangerous effects of these compounds on public health imply the need to regulate the allowed amounts of these compounds present in drinking water.

Another difference between the nitrogen-containing DBPs and the earlier DBPs is the polar nature of the compounds. The DBPs discovered in the 70’s are highly polar, while the newly discovered N-DBPs are only semi-polar. This presents a problem because polar compounds cannot be removed from the polar environment of water. Thus, scientists are forced to derivatize the compounds before extraction to make them non-polar. Derivitization has many drawbacks because it creates chemical waste, adds cost to extraction, and is time consuming.

Another difference in the compounds is the volatile nature of the two classes. The original class of DBPs is highly volatile, whereas the newly discovered nitrogen-containing DBPs are semi-volatile. The semi-polar and semi-volatile nature of the nitrogen-containing DPBs makes them more difficult to extract from water and thus more difficult to quantify.

One of the biggest obstacles to quantifying polar/semi-polar and semi-volatile DBPs in water is extracting the DBPs from the water for analysis on a Gas Chromatograph/Mass Spectrometer (GC/MS). Currently, the EPA uses Solid Phase Extraction (SPE) and Liquid-

Liquid Extraction (LLE) as standard extraction methods for drinking water analysis (Environmental Protection Agency 2006). LLE is a process of extraction from a polar solvent, such as water, containing nonpolar molecules by adding an immiscible nonpolar solvent. Partition occurs between the two layers; the nonpolar molecules within the water are more soluble in the nonpolar solvent and partition into the nonpolar solvent. LLE is a reliable method that is used to extract many compounds, but it creates large amounts of harmful environmental waste and is labor intensive (Handley 1999).

SPE is the most common method of extraction used in environmental chemistry. It is a better method because it creates less harmful waste and is quicker than LLE. SPE is performed by passing water through a short column that is packed with a nonpolar solid phase. The nonpolar compounds in the water have an affinity for the solid phase and adhere to it. After passing the water through the column, a nonpolar solvent is passed through the column and the nonpolar compounds desorb from the nonpolar solid. The nonpolar solvent containing the nonpolar compounds can then be analyzed (Harris 2007).

These methods are widely used for water analysis, but they are lengthy, create chemical waste, and are not very cost efficient. An alternative method has recently been developed that reduces chemical waste, cost, and time. This method is called Solid Phase Micro-Extraction (SPME), is a relatively new method and little, if any, research has been done on applying it to N-DBPs. Research has been done to determine the application of SPME on the DBPs discovered in the 1970s (Sarrión 2000). The use of SPME could ease the difficulty of extracting analytes from water and serve as a single extraction method for detecting multiple classes of DBPs simultaneously in drinking water.

4. Solid Phase Micro-Extraction

Solid Phase Micro-Extraction (SPME) was first introduced in 1989 but is slowly gaining attention by the EPA as a possible standard method of extraction. SPME utilizes a fiber that is coated with a liquid polymer, a solid sorbent, or a combination of both. The fiber is placed directly in the sample or in the headspace of the sample and removes the analytes from the sample by absorption or adsorption. The analytes from the sample can then be analyzed via Liquid Chromatography (LC), LC/MS, GC, or GC/MS. For the research presented here a GC/MS is used, and the fiber is placed in the injection port, which is set at a temperature above the boiling point of the analytes, to remove the analytes from the fiber. The high temperature transfers the analytes into the gaseous phase where they are carried into the column of the GC by a carrier gas. When using SPME, several parameters must be optimized to obtain the optimal extraction of analyte from a sample.

4.1 Fibers

SPME fibers are the backbone of the extraction process. One fiber can be reused for up to 100 analyses depending on care and application. Each fiber has a polymer coating that can be a variety of types and thickness. Polydimethylsiloxane (PDMS) and Divinylbenzene (DVB) are two of the coating types available for SPME. They differ by the exposed functional groups that allow different interactions between the analytes and the fibers to occur. The fibers can also differ by the support to which the coating is affixed. The coating can be affixed to supports of silicon (glass), carboxen, and a variety of other supports. Table 1 shows the different types of fibers available for purchase from Sigma Aldrich, a major chemical supply company and only supplier of SPME fibers. The criterion for the type and thickness to use depends on the

molecular weight and the polarity of the compounds. Each fiber optimally extracts a different type of analyte and picking the right fiber is essential. Table 2 is the Supelco fiber selection guide, which shows each type of fiber and the analytes that are optimally extracted by it.

Table 1:

StableFlex Kit: 65 µm PDMS/DVB, 50/30 µm DVB/Carboxen/PDMS, 85 µm Carboxen/PDMS, and 85 µm polyacrylate coated fibers

Kit 1 (For Volatiles and Semi-volatiles): 85 µm polyacrylate, 100 µm PDMS, and 7 µm PDMS coated fibers.

Kit 2 (For Volatile or Polar Organics in Water): 75 µm Carboxen/PDMS, 65 µm PDMS/DVB, and 85 µm polyacrylate coated fibers.

Kit 3 (For SPME/HPLC Analysis): 60 µm PDMS/DVB, 85 µm Polyacrylate, and 100 µm PDMS coated fibers.

Kit 4 (For Flavors and Odors): 100 µm PDMS, 65 µm PDMS/DVB, and 75 µm Carboxen/PDMS coated fibers.

Kit 5 (For Flavors and Odors): 100 µm PDMS, 65 µm PDMS/DVB, 85 µm Carboxen/PDMS, and 50/30 µm DVB/PDMS coated fibers.

Table 2:

Sepelco Fiber Selection Guide

Analyte Type (Molecular Weight)	Recommended Fiber
Gases and low molecular weight compounds (MW 30-225)	75 µm/85 µm Carboxen/polydimethylsiloxane
Volatiles (MW 60-275)	100 µm polydimethylsiloxane
Volatiles, amines and nitro-aromatic compounds (MW 50-300)	65 µm polydimethylsiloxane/divinylbenzene
Polar semi-volatiles (MW 80-300)	85 µm polyacrylate
Non-polar high molecular weight compounds (MW 125-600)	7 µm polydimethylsiloxane
Alcohols and polar compounds (MW 20-275)	60 µm Carboxen (PEG)
Flavor compounds: volatiles and semi-volatiles, C3-C20 (MW 40-275)	50/30 µm divinylbenzene/Carboxen on polydimethylsiloxane on a Sableflex fiber
Trace compound analysis (MW 40-275)	50/30 µm divinylbenzene/Carboxen on polydimethylsiloxane on a 2 cm Sableflex fiber
Amines and polar compounds (HPLC use only)	60 µm polydimethylsiloxane/divinylbenzene

4.2 Stirring/Agitation

Agitation or stirring the sample can increase the amount of analyte extracted for some compounds. Agitation shortens equilibration time for the analyte between the aqueous phase and the organic fiber phase and improves accuracy and precision. This is crucial when analyzing semivolatile compounds by immersion sampling. It is essential to maintain a consistent agitation rate for every extraction to ensure good precision (SPME 2001). Suitable methods for agitation of the sample include stirring, sonication, and vibration. The research presented here will focus on stirring with a Teflon® stir bar.

4.3 Temperature

The temperature of the sample is critical for accurate quantitation and good precision. Heating or cooling may be necessary to achieve optimal extraction because of the effects of the temperature on both the fiber and the sample. If the temperature is too high with SPME, the analytes can be driven off of the fiber, which reduces the overall sensitivity (SPME 2001). Generally, heating samples is not needed for extraction by immersion. For some applications with non-volatile or high boiling semivolatile compounds, a small amount of heat applied to the sample can shorten the equilibration time. The research presented here will focus on the use of a water bath to control the temperature of the sample.

4.4 Extraction time

Extraction time is defined as the amount of time that the fiber is placed in the sample to allow equilibration between the analyte and the fiber. Extraction time can vary from 30 seconds to an hour, but most extractions typically take only 15-20 minutes (SPME 2001). The extraction

time varies with the molar mass of the compounds, the fiber coating and thickness, the type of extraction (headspace vs. immersion), and sample concentration.

4.5 Holding time

Holding time is defined as the amount of time between the collection of the sample to the time that it is analyzed. This is important because many analytes can be affected in a variety of ways during the holding time. Many times the holding time is set to prevent the loss of analyte from degradation. Current standards call for maximum holding times for anywhere from 7-14 days depending on the analytes (Greenberg 1992). To complete a standard method for these compounds a holding time study must be conducted and a maximum holding time must be determined.

5. Gas Chromatography / Mass Spectrometry (GC/MS)

Chromatography is the process of separating a mixture based on the mixture's components and properties. Some types of chromatography include column chromatography, thin layer chromatography, gas chromatography, high pressure liquid chromatography, affinity chromatography, and a variety of other techniques. Each chromatography technique has a specific process by which it separates analytes from their respective solvents. The research presented here used gas chromatography to separate the compounds of interest.

Mass spectrometry is a technique that measures the masses of molecules or fragments of molecules. Analytes are ionized, separated according to their mass-to-charge ratio (m/z), detected, and then recorded. Mass spectrometers are composed of an ion source, where ionization occurs, an analyzer, where the separation by m/z occurs, and a detector, where the

ions are converted into an electrical signal for display in the computer (Harris 2007). There are a variety of mass spectrometer analyzers, including but not limited to ion trap analyzer, double focus mass spectrometer, and quadrapole mass analyzer. For the research presented here, a quadrapole mass analyzer was used.

The GC and the MS may seem unrelated, but when the MS is connected to the GC, it is a relatively inexpensive method of separating and identifying compounds. The linked process is called GC/MS and is an excellent process for determining specific compounds found within a sample. While the GC can facilitate identification of analytes within a sample, the MS confirms the identity of an analyte at a particular time in the chromatogram. By correlating the GC and MS data, each separated analyte can be identified and quantified.

5.1 Gas Chromatography

Gas chromatography operates on the principle of partitioning. The sample is injected into a port through a septum. The port is heated to the desired temperature, which should be higher than the analyte's boiling point to ensure that the analyte enters the gaseous phase and travels through the column. Once the analyte is in the gaseous phase, it is carried along by a carrier gas, which is typically an inert gas such as helium, and is carried into the column.

The column is a hollow tube that has a small diameter (0.1 to 0.6 mm) and has a sufficient length (15 to 100 m) to allow partitioning. The most common size of column is 30 m in length. The column used in these experiments was a porous-layer open tubular column (PLOT), which has a solid stationary phase affixed to the inner wall of the column (Harris 2007). As the analyte travels through the column, partitioning occurs, and the solute equilibrates between the mobile phase and the stationary phase.

As the temperature rises in the GC oven that houses the column, the analyte absorbs energy, and when enough energy is absorbed, the analyte goes back into the mobile phase and is carried to the detector, where the abundance of analyte is measured by some type of detector. There are various types of detectors for GC, including flame ionization detector (FID), electron capture detector (ECD), thermal conductivity detector (TCD), and mass spectrometer (MSD). An MS detector was used for the research presented here. Figure 2 displays a representative chromatogram from a GC/MS instrument.

5.2 Mass Spectrometry (MS)

As stated before, the mass spectrometer's major component is the analyzer, and the quadrupole was the analyzer used for the research presented here. The quadrupole mass analyzer uses a direct current (DC) and an alternating radiofrequency (RF) to separate the ions. The gaseous sample enters an ionization chamber consisting of a hot filament that emits electrons, which are accelerated through a 70V electric potential, leading to high-energy electrons. The incoming sample particles interact with the high energy electrons, leading to ionization and fragmentation of the analytes present in the sample. There is a repeller plate that carries a small positive potential to push the positively-charged ions toward the analyzer. The quadrupole then separates the ions by using rapidly varying voltages to select different ions of different masses to reach the detector. If the correct voltage is not chosen, then the ions will not have a stable trajectory to go through the quadrupole and reach the detector. Instead, the ions will collide with the quadrupole and undergo neutralization. Thus, the computer software scans the voltages to successively allow ions with different m/z values to reach the detector, leading to a mass spectrum as shown in Figure 3.

```
File        : D:\DATA08\052008C.D
Operator    : JWQ
Acquired    : 20 May 08  11:11      using AcqMethod N-DBP
Instrument :   GC/MS Ins
Sample Name: N-DBP ~50ug/mL
Misc Info   :
Vial Number: 1
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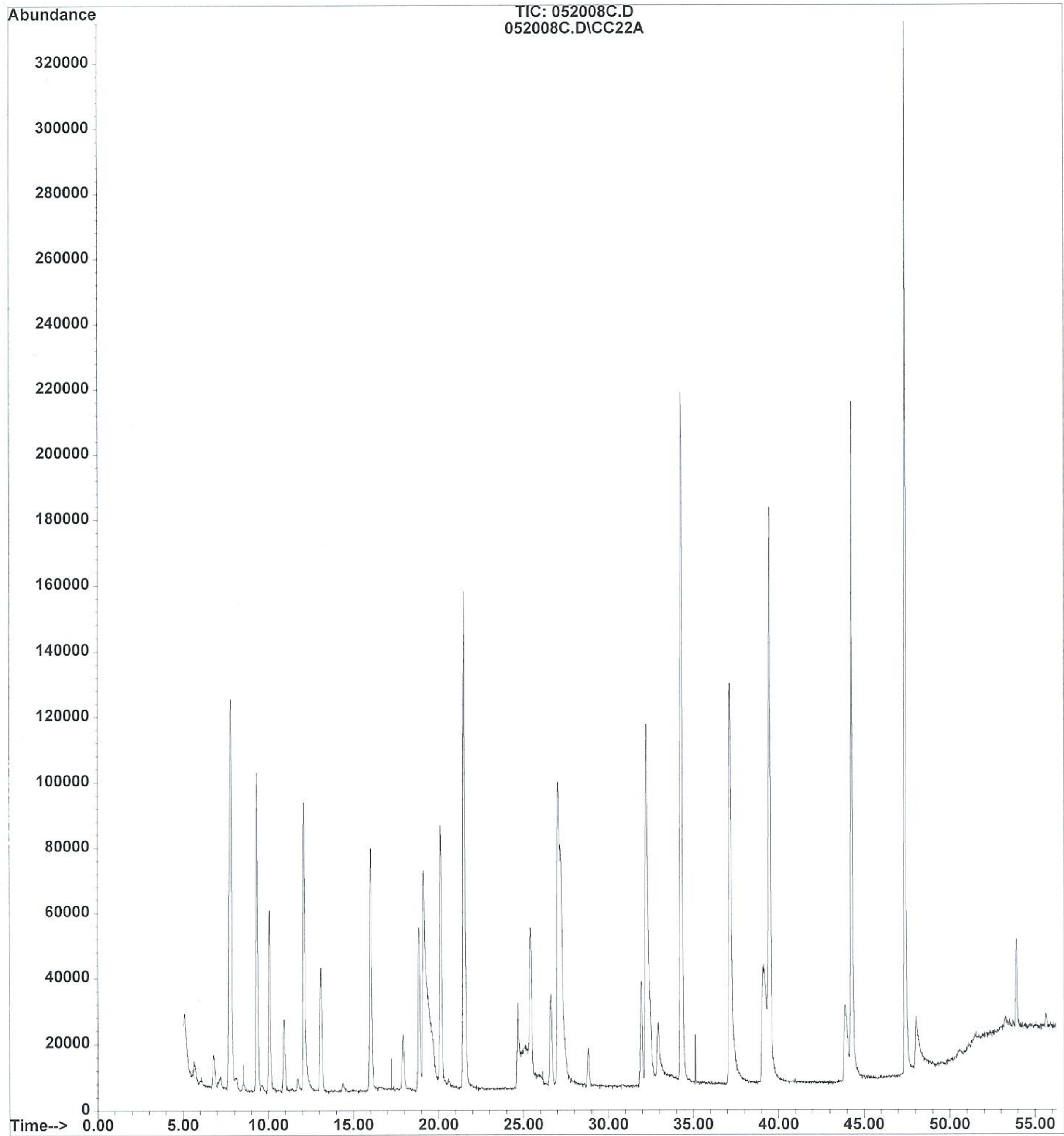


Figure 2: A representative chromatogram of a mixture of N-DBPs

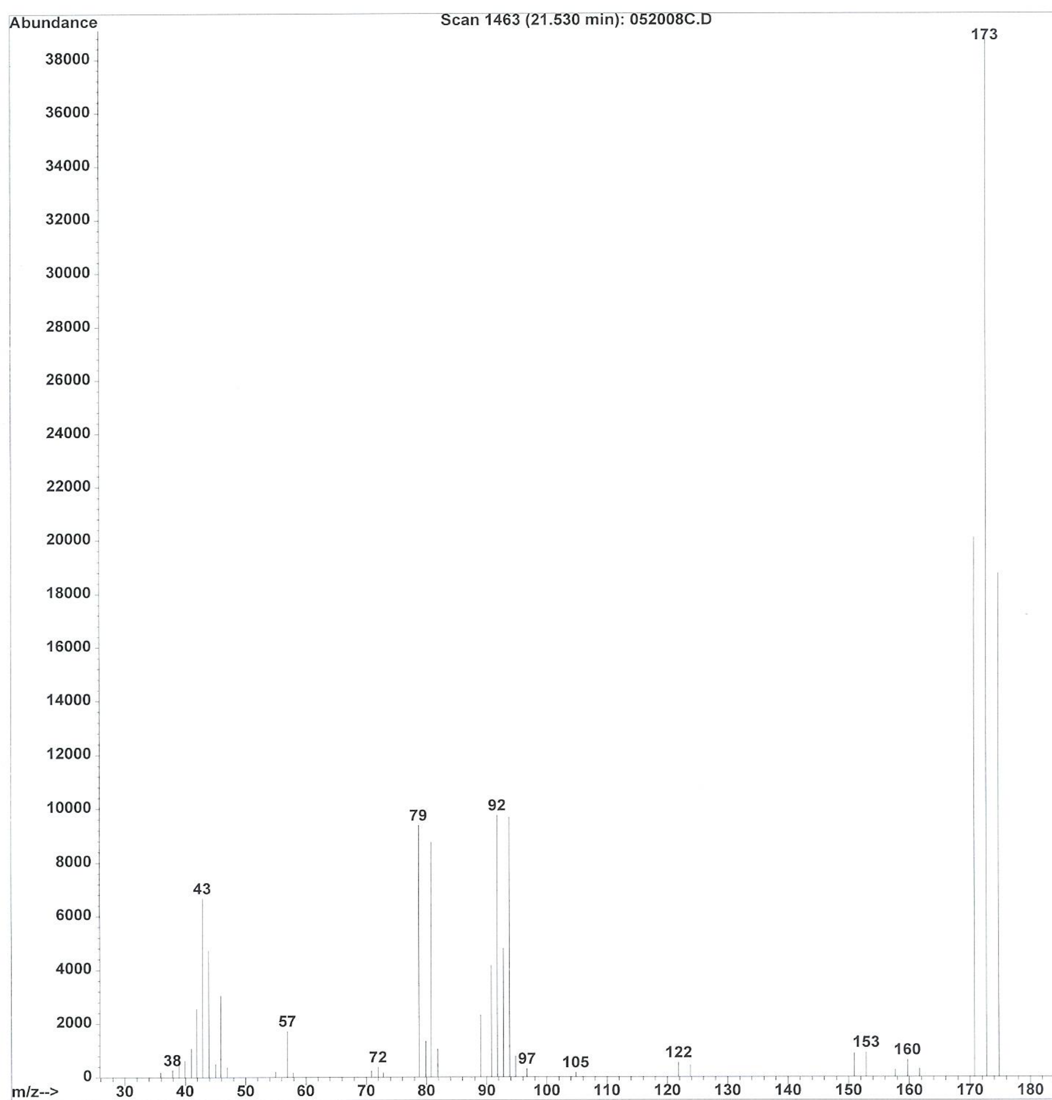


Figure 3: A representative mass spectrum of Dibromoacetonitrile.

6. Project focus

The research presented here focuses on determining the conditions for optimal extraction of haloacetonitriles, halonitromethanes, and haloacetamides from drinking water. To find the optimal conditions for the extraction of these types of compounds, several parameters surrounding typical SPME extraction techniques were evaluated. The parameters that were evaluated include the use of an internal standard, extraction time, stirring/agitation, fiber type, holding time, and temperature. The goal of this research is to develop an analytical method to use as a standard method for extracting these types of compounds from drinking water.

MATERIALS AND METHODS

1. Gas Chromatography/Mass Spectrometry

An Agilent 5890 Series II GC with a 50 m x 0.32 mm x 0.68 µm methyl phenyl column was used. An Agilent 5972 Series Mass Selective Detector with an electron ionization source and a single quadrupole analyzer was used. Each day, an instrument performance check was completed on the GC/MS by running an instrument auto tune and analyzing a sample of 50 µg/mL DFTPP (decafluorotriphenyl phosphine, Supelco®, 97.5% purity) in acetone. An Excel spreadsheet was prepared to analyze the ratios of the MS peaks for the DFTPP and to ensure that the GS/MS is working within the range set by EPA standards (Greenberg 1992). The Excel spreadsheet and EPA standards for DFTPP can be found in Appendix A. Once the spreadsheet confirmed the performance of the instrument, the sample analysis scheduled for the day began.

The appropriate GC temperature program was developed by preparing individual samples of each analyte at a concentration of 100 µg/mL and analyzing them at first individually then in a solution containing all of the analytes to determine their retention times and mass spectra. This information was put into a Library for reference of each compound and can be found in Appendix C. The temperature program was varied until all of the analytes peaks were resolved and easily identified. The final temperature program used for all samples is shown in Appendix B, resulting in a GC runtime of 56.17 minutes.

Once the analyte has been extracted using SPME, the SPME holder is inserted into the GC and the fiber is exposed. The appropriate temperature program is started upon fiber insertion into the injector port. After 1 minute of exposure the fiber is retracted and removed from the injection port. Upon completion of the temperature program, the data is saved automatically to the hard drive for later interpretation.

2. Sample Preparation

The research presented here focused on the nitrogen-containing DBPs haloacetonitriles, halonitromethanes, and haloacetamides. Although the haloacetamides were not evaluated for each parameter of SPME the standard solutions were made in similar fashion to the haloacetonitriles and halonitromethanes. Samples were prepared using standards received from the EPA and were used without further purification. Table 3 is a list of the compounds used in the experiment and their purities.

Standard solutions of each of the compounds being analyzed were made by dissolving a pure solid or diluting a pure stock solution in methanol. Samples were prepared in amber vials to protect the samples from UV light and possible chemical degradation. Standard solutions were made by one of two methods. To determine the concentration of pure liquid compounds, 1 mL of methanol was injected into a vial and weighed. Then, 1 μL of the liquid compound was injected into the vial, and the mass difference was used as the concentration of the solution in mg/mL. To determine the concentration of pure solid compounds, the compounds were weighed in the amber vials, and then 1 mL of methanol was added to the solid. The mass of the solid determined the concentration of the solution in mg/mL

Samples prepared for SPME contained 8 mL of High Pressure Liquid Chromatography or HPLC-grade water with a desired concentration of analyte in a 10 mL glass vial and closed by a crimp top cap with a silicone septum. The samples were prepared by adding the calculated amounts of nitrogen-containing DBPs and diluting to 8 mL with HPLC grade water.

Table 3: List of Experimental Compounds

Compound Name	Purity	Amount Recieved	Molecular Formula	Molar Mass
Chloronitromethane	90+%	200 mg	$ClCH_2NO_2$	95.49
Dichloronitromethane	95+%	200 mg	Cl_2CHNO_2	129.93
Bromochloronitromethane	85%	200 mg	$BrClCHNO_2$	174.38
Bromodichloronitromethane	90+%	200 mg	$BrCl_2CNO_2$	208.83
Dibromonitromethane	90+%	200 mg	Br_2CHNO_2	218.83
Dibromochloronitromethane	90+%	200 mg	Br_2ClCNO_2	253.28
Tribromonitromethane	95+%	1g	Br_3CNO_2	297.73
Iodoacetonitrile	98%	1g	ICH_2CN	292.84
Tricholoracetonitrile	98%	1g	Cl_3CCN	144.39
Bromoacetonitrile	97%	1g	$BrCH_2N$	119.95
Chloroacetonitrile	99%	1g	$ClCH_2N$	75.50
bromodichloroacetonitrile	90+%	200 mg	$BrCl_2CN$	188.84
tribromoacetonitrile	90+%	200 mg	Br_3CN	277.74
dichloroacetonitrile	99%	1.3 g	Cl_2CHCN	109.94
Bromochloroacetonitrile		100 μg/mL	$BrClCHCN$	154.39
Dibromoacetonitrile		50 mg	Br_2CHCN	198.84
Bromoiodoacetonitrile / Diiodoacetonitrile	50% BrI 29% diBr 21% diI	1g	$BrICHCN$	245.84
			I_2CHCN	292.84
Tribromoacetamide	>95%	103 mg	Br_3CCONH_2	295.76
2-Chloroacetamide	-	1g	$ClCH_2CONH_2$	93.51
Bromoacetamide	98%	1g	$BrCH_2CONH_2$	137.96
Iodoacetamide	-	1g	ICH_2CONH_2	184.96
2,2,2-trichloroacetamide	99%	1g	Cl_3CCONH_2	162.40
2,2-dichloroacetamide	98%	1g	$Cl_2CHCONH_2$	127.96
2,2-dibromoacetamide		200mg	$Br_2CHCONH_2$	216.86
bromodichloroacetamide			$BrCl_2CCONH_2$	206.85
Chloroiodoacetonitrile		209mg	$ClICHCONH_2$	219.41
Bromoiodoacetonitrile		70.8mg	$BrICHCONH_2$	263.86
Diiodoacetamide		70mg	$I_2CHCONH_2$	310.86
Bromochloroacetamide		100mg	$BrClCHCONH_2$	172.41
Dibromochloroacetamide		201mg	$Br_2ClCCONH_2$	251.30438

3. Solid Phase Micro-Extraction – SPME

The research presented here focused on determining the conditions for optimal extraction of halonitromethanes, haloacetonitriles, and haloacetamides from drinking water. To find the optimal conditions for the extraction of these types of compounds, several aspects of typical SPME extraction techniques were evaluated. The parameters that were evaluated are an internal standard, extraction time, stirring/agitation, fiber type, holding time, and temperature. For each parameter under investigation, three samples were analyzed to check precision.

3.1 Fiber Type

To determine the type of fiber with the best ability to extract the nitrogen-containing DBPs from the solution, three samples were analyzed using each of the two fibers with an extraction time of 20 minutes at room temperature and no stirring. The two fiber types evaluated were – a 60 µm Carboxen/Polydimethylsiloxane (PDMS) versus a 65 µm PDMS/Divinylbenzene (DVB). The two coatings are very different in structure and composition as can be seen in Figure 4. Both of these fibers work on the basis of adsorption, which is explained in a paper by the only makers of SPME fibers, Supelco.

> Adsorbent type fibers extract analytes by physically interacting with the analytes. Adsorbents are generally solids that contain pores or high surface areas. The extraction can be accomplished by trapping the analytes in internal pores. These micro- and meso- pores are ideal for trapping small and midsized analytes and usually retain the analytes until either energy is applied or they are displaced by a solvent. Macropores, primarily on the surface of the material, can also trap larger analytes, but generally retain the analytes through hydrogen bonding or van der

Waals interactions. Because there are a limited number of sites, the analytes can complete. This can result in reduced capacity and/or displacement of analytes with low distribution constants by those with higher distribution constants (Shirey 1999).

Although these two fibers are adsorptive fibers they differ in many ways. The Carboxen-PDMS differs from the PDMS/DVB in the percentage of micropores. The Carboxen-PDMS has a greater percentage of micro pores as opposed to the PDMS/DVB which has a greater percentage of macropores. Since these compounds under investigation are mid to large sized molecules, it is postulated that the PDMS/DVB will be more effective at extracting these compounds. The research was completed by analyzing samples of the same concentration of DBPs with each of these two fiber types. The abundances were calculated and compared to one another to determine which fiber successfully extracted more of the analytes from the water.

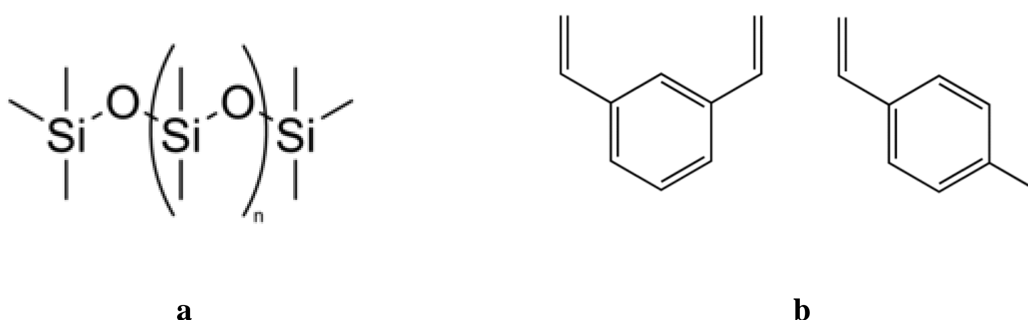


Figure 4: Two different SPME fiber coatings: a) Polydimethylsiloxane (PDMS) and b) Divinylbenene (DVB).

3.2 Extraction Time

Extraction time is an extremely important piece of information to know when extracting analytes from a solution. To find the optimal extraction time for the nitrogen-containing DBPs,

water samples were prepared as stated in section 2, and the SPME fiber was left in the sample for different time intervals. The optimal extraction time was determined by a bracketing method where the time intervals of 20, 60, and 70 minutes were tested, and the time was narrowed based on the results. The time that resulted in the greatest signal for the analytes detected by the GC/MS was considered the optimal extraction time.

3.3 Sample Stirring/Agitation

Stirring is another factor that can aid in optimal extraction of an analyte from solution and can be easily tested. There are several methods of sample stirring or agitation that can be used and using a stir bar is one method that is generally practiced. Using a stir bar raises questions as to whether the analyte being extracted is adhering to the stir bar and decreasing extraction efficiency. To test the effect of the stir bar on the extraction process, several extractions were done with and without the stir bar. Next a stir bar was added to three samples without stirring and were extracted and tested. This experiment determined whether the stir bar affects extraction through the analytes adhering to the stir bar and lowering extraction efficiency.

Since the results showed that the stir bar did not decrease efficiency, then another set of experiments were performed to determine the effect of the speed of stirring on extraction. A Thermolyne Cimarec® 1 stir plate was used to perform the stirring throughout this experiment. The optimal extraction speed was determined by a bracketing method, where the rotations per minute (rpm) were tested at various intervals, and the speed was narrowed based on the results and eventually pinpointed to an exact speed. The speed that resulted in the greatest signal of analytes detected by the GC/MS was considered to be the optimal extraction speed.

3.4 Holding Time

The holding time study was conducted over a 21 day period. Three samples were prepared for each time period tested. The samples consisted of an 8 mL water sample with a concentration of 50 μg/mL and were placed in a 10 mL glass vial and closed with a crimp top cap with a silicon septum. All samples were prepared at the same time and left to sit over a period of 1 day, 7 days, 14 days, and 21 days. The time intervals were based off of current standard methods which are generally either 7 or 14 days maximum holding time (Greensberg 1992). After the samples sat for the appropriate time, the analytes were extracted using SPME and were analyzed using the GC/MS.

3.5 Temperature Control

Temperature control is very important in having accurate, precise, and reproducible results. Heating or cooling a sample can change the amount of analyte extracted by SPME. This poses a problem when performing extractions because our laboratory temperature fluctuates from day to day. To keep temperature fluctuations from affecting the extraction, the samples were kept at a constant temperature during the extraction process by using a water bath. Performing extractions at a constant temperature reduces any error in the extraction process. Since heating compounds can also make extraction quicker and more effective, extractions were tested at room temperature (RT), which was 25ºC, and 30ºC.

3.6 Internal Standard

The internal standard is added to a sample in a known amount to quantify the analyte. The internal standard being used in this research is bromoacetamide-2-13C. The carbon-13 gives

a different MS signal than bromoacetamide without C-13 and can be easily identified using the MS. The concentration of the analyte can be calculated using the response factor as shown in Equation 1. Before each sample is subjected to SPME, the internal standard will be added to the water sample to give a concentration of 25 μg/mL. This concentration should produce a strong signal in the GC/MS but not be too high to allow other analytes to be detected.

$$\frac{\text{Area of analyte signal}}{\text{Concentration of analyte}} = F\left(\frac{\text{area of standard signal}}{\text{concentration of standard}}\right)$$

Equation 1: Response Factor of Internal Standard

Thus, if the instrument is not working properly, the internal standard signal will decrease in a similar way that the analyte signal will decrease. The response factor adjusts for the decrease allowing direct comparison from day to day and run to run. The use of an internal standard was evaluated by comparing calibration curves calculated both with and with the presence of the internal standard.

RESULTS AND DISCUSSION

In this study the parameters of SPME were tested to find the optimal conditions of extraction for haloacetonitriles, halonitromethanes, and haloacetamides. The parameters studied include the fiber type, temperature, extraction time, sample stirring/agitation, and holding time. For each of the parameters tested, three samples of the compounds at the same concentrations were evaluated to obtain a precise measurement.

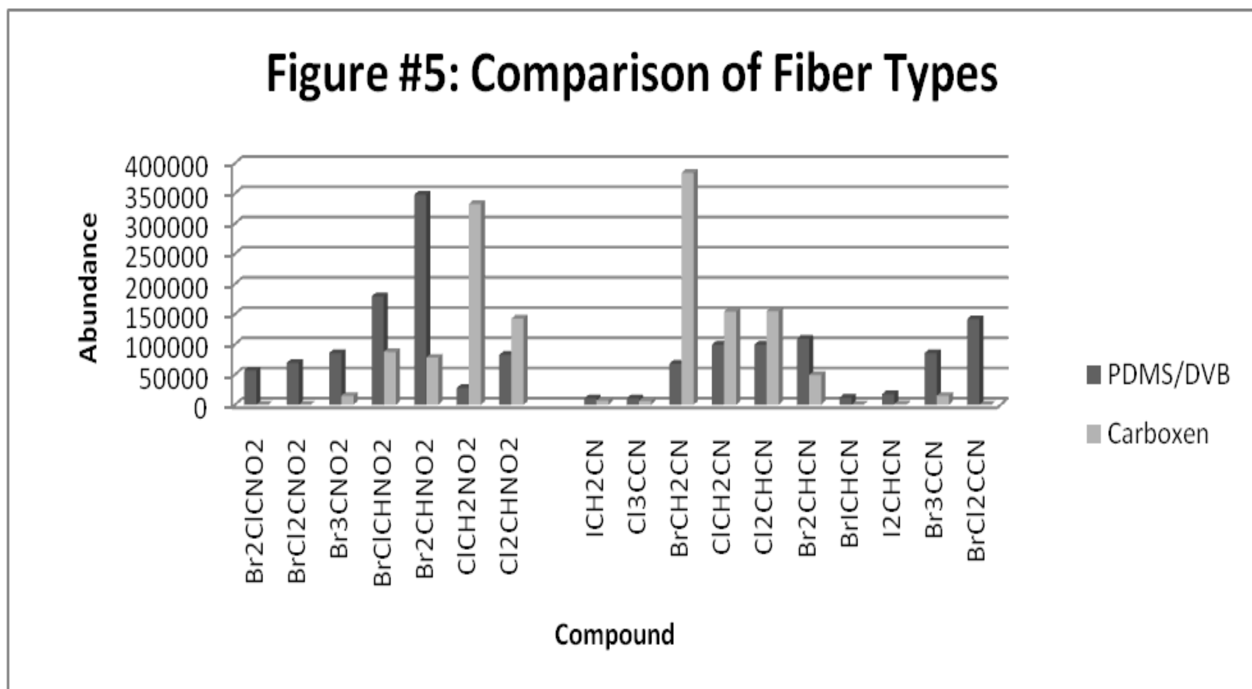
1. GC/MS Program

Before any of the experiments involving SPME could be performed, the GC/MS had to have an appropriate temperature program, and a library of the compounds had to be constructed. The temperature program was crafted by changing the program until the compounds produced a definable signal. The rate was changed to resolve the compounds that had similar retention times to make the compounds discernable in the GC. A printout of the full temperature program and GC/MS settings can be found in Appendix B.

Once a temperature program was established that encompassed all of the compounds being tested, a concentrated sample of each compound was injected on the GC/MS. This allowed both the retention time in the GC and the spectrum of the MS for each compound to be identified and recorded. This was important because tests involving multiple compounds from the same class would produce many peaks in the GC and make identification of specific compounds difficult without a reference. The library of haloacetonitriles and halonitromethanes as well as a composite chromatogram which has each compound present can be found in Appendix C.

2. Fiber type

Two fibers containing different materials were investigated to determine the best fiber to extract all of the compounds under investigation. The two fibers tested included a PDMS/DVB fiber and a Carboxen/PDMS. Each of these fiber types was very different in its effectiveness to extract the compounds from the water. The PDMS/DVB was more effective at extracting 12 of the 17 compounds than was the Carboxen/PDMS fiber as can be seen in Figure 5. The PDMS/DVB fiber is more effective due to the size of the compounds and decreased percentage of micropores. It can be seen in Figure 5 that the Carboxen/PDMS fiber was more effective at extracting the 5 compounds with the smallest molecular weights. This is to be expected due to the Carboxen/PDMS's increased percentage of micropores that allow more of the smaller compounds to adsorb to the fiber. Since the aim of this project was to find the optimal extraction for the compound class as a whole, it was determined that the PDMS/DVB fiber was more effective at extracting the nitrogen containing DBP's and was used as the extraction fiber for the remaining tests.

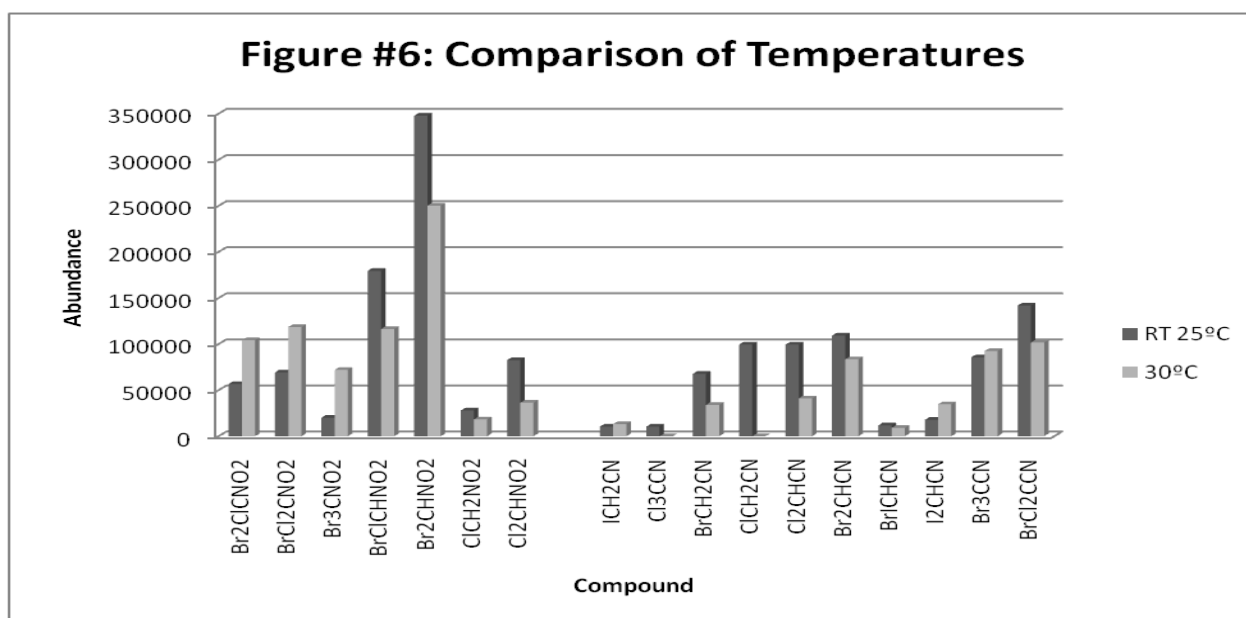


3. Temperature

Because the temperature of a sample can easily affect the concentration of compounds, it is important to maintain samples at or below a certain temperature. The temperatures tested were room temperature (RT) of 25ºC and an increased temperature of 30ºC.

It was determined that extracting the compounds from a sample at a temperature of 25ºC allowed a greater abundance of the analyte to be extracted than that of a sample maintained at a temperature of 30ºC. As can be seen in Figure 6, greater abundances were obtained at 25ºC for 11 of the 17 compounds tested.

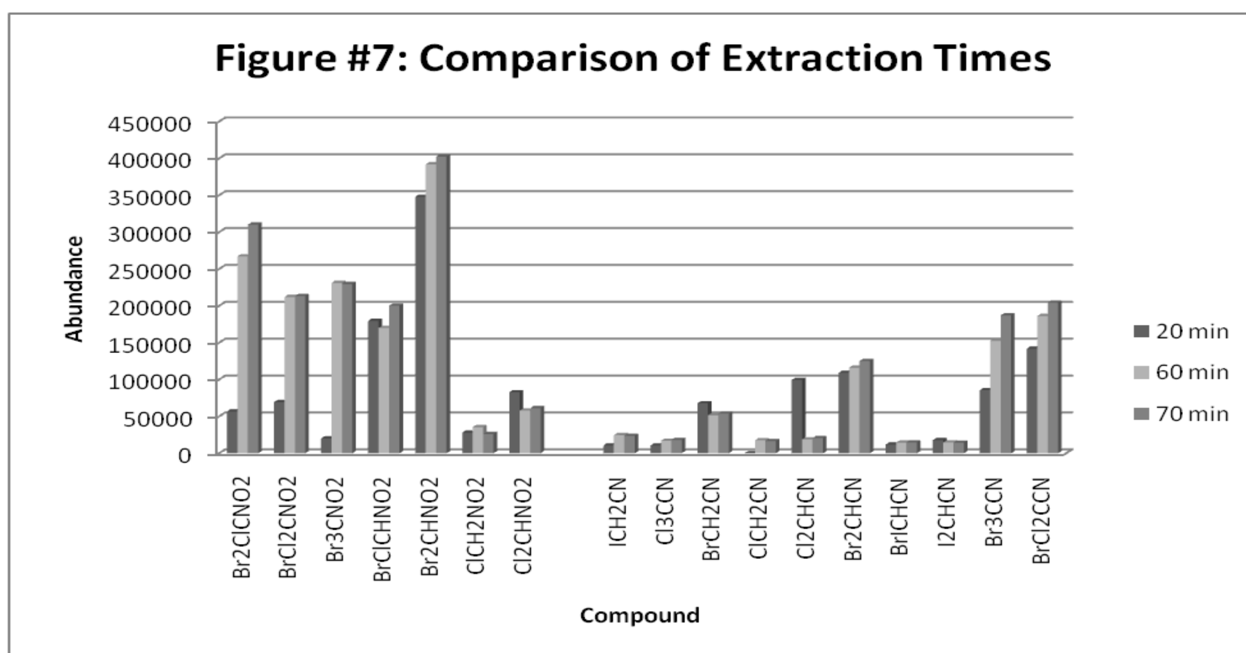
Because these compounds are semi-volatile, an increase in temperature of the solution adds energy to the compounds, possibly causing a partitioning of the analytes from the liquid phase to the gas phase. If the semi-volatile compounds partition into the headspace of the sample, then there is a decreased concentration of analytes in the liquid phase of the sample to be extracted via SPME. The decreased concentration causes the extraction to be less efficient and produce a lower abundance on the GC/MS. Thus, solutions were maintained at room temperature (25ºC) when performing the remaining extractions.



4. Extraction Time

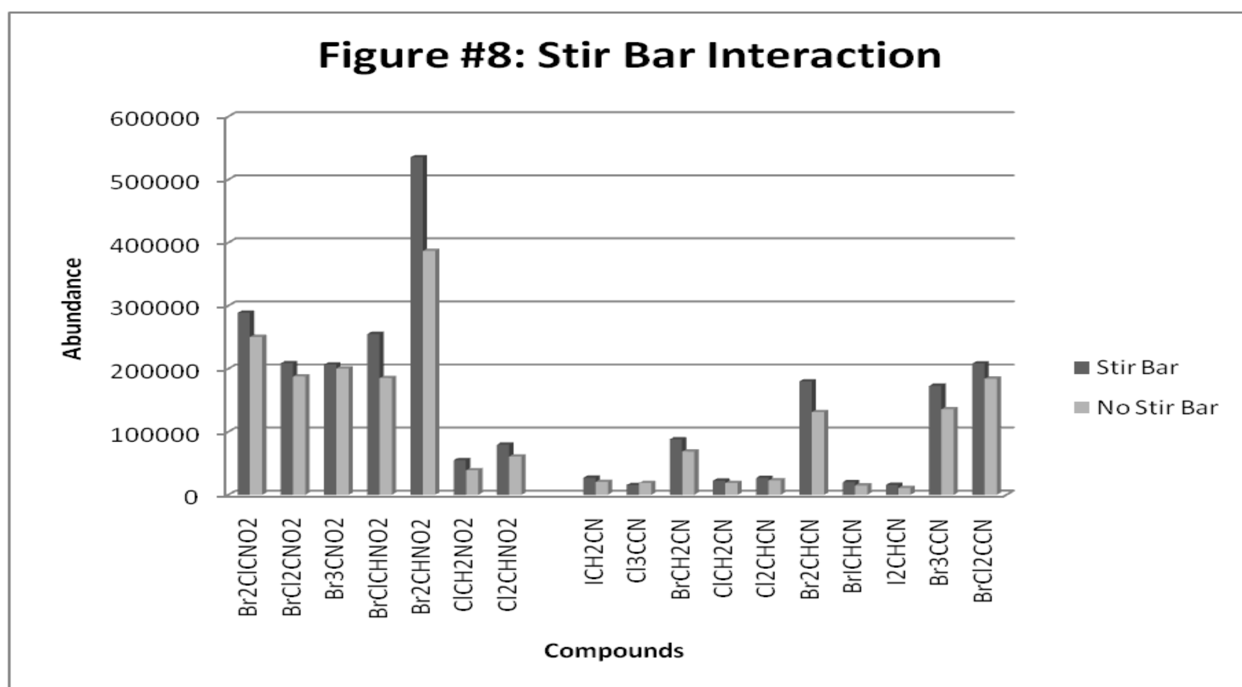
Because SPME relies on interactions between the SPME fiber and the analytes in the compound, the extraction time or amount of time that the fiber is exposed to the sample is very important in finding the optimal conditions for extraction. It takes time for the analytes to diffuse to the surface of the fiber and even more time for the analytes to absorb/adsorb into/onto the fiber.

Both a 60-minute and 70-minute extraction time produced similar results. Figure 7 shows that a 70-minute extraction time produced higher abundances for the majority of the compounds and should in fact be determined to be the optimal time for extraction. The standard deviation, however, was higher for the compounds when a 70-minute extraction time was used than when a 60-minute extraction time was used. Although a 70-minute extraction time produced greater abundances in the majority of the compounds it also had a higher standard deviation, thus an extraction time of 60-minutes was determined to be the optimal extraction time for this class of compounds.



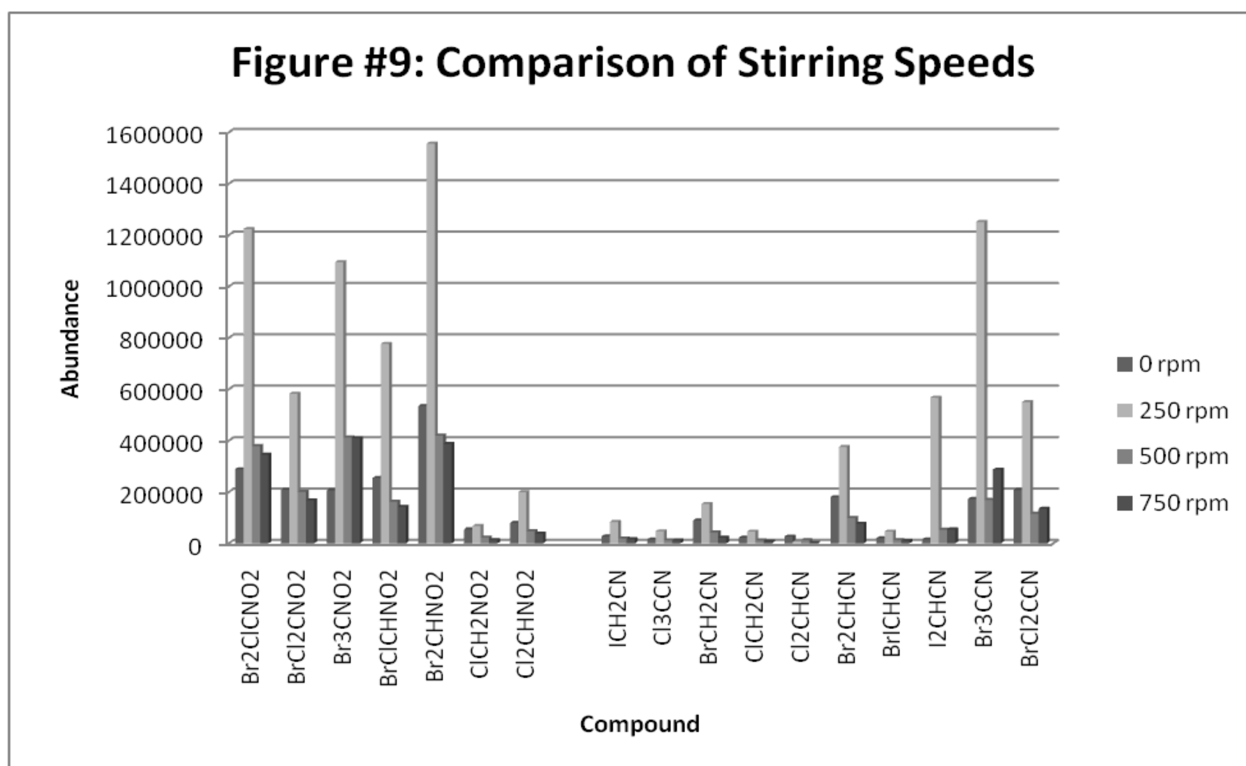
5. Sample Stirring / Agitation

SPME is a method that relies on the interaction between the analytes and the SPME fiber. This interaction can be increased by stirring the sample and causing the analytes to be in contact with the fiber. Originally sonication was going to be used to agitate the sample. However, upon the first attempt of sonication, the fiber was damaged and the fiber could not be placed in the GC port without leaving behind debris. It was determined that sonication was not a viable method of agitation for this particular fiber and that another method of stirring had to be tested. The next method of stirring investigated was the use of a Teflon® stir bar. Before stirring of the sample was tested with the bar, it first needed to be established if the stir bar would interact with the analytes in the sample and affect the amount of analyte extracted by the SPME fiber. As can be seen in Figure 8, when a Teflon® stir bar is added to a sample for 60 minutes it does not statistically affect the extraction of the compounds using SPME. This proves that the Teflon® does not adsorb or interact with any of the compounds studied.



The next course of action was to determine the optimal stirring speed using a stir plate. Stirring speeds of 0, 250, 500, and 750 rpm were tested to determine which setting gave the highest abundance for the compounds. In Figure 9, it can be seen that a stirring speed of 250 rpm resulted in higher abundances for all of the analytes as compared to 0, 500, and 750 rpm. The abundances of the 0, 500, and 750 rpm speeds as compared to 250 rpm is significantly different and increases the amount of analyte extracted significantly.

A speed of 250 rpm as the optimal extraction speed is due to equilibration time and laminar flow of the sample. The speeds affect the amount of time that the analytes actually spend in contact with the fiber. At higher speeds there seems to be an increase of turbulent flow. This turbulent flow, which can prevent the compounds from diffusing to the fiber surface, appears to be very significant at speeds of 500 and 750 rpm. At a speed of 250 rpm, however, it appears that it can be classified as laminar flow and the speed allows adequate diffusion to the fiber surface. Thus, a stirring speed of 250 rpm was used for all remaining SPME extractions.



6. Holding Time

Because these compounds can degrade over time, a holding time study is pertinent to determining the standards for this class of compounds. The holding time study was conducted with an internal standard of Tribromonitromethane C-13. The internal standard was added to the samples at day 0 when it should have been added just before extraction at each time interval. Table 4 shows the data from the internal standard and the inconsistency observed due to the improper addition. The study did prove however that the internal standard does degrade with time in a mixture with other N-DBPs and is significant to its ability to be used as a surrogate compound. No further data analysis was conducted once it was determined that the study was not conducted properly.

Table 4: Holding Time Internal Standard Data

	Tribromonitromethane C13				
Day	**INST1**	**INST2**	**INST3**	**AVG**	**STDEV**
1	61736	44448	43040	43744	995.6063
7	10157	7248	2526	4887	3338.958
14	14911	58560	75912	49794.33	31431.01

7. Internal Standard

The internal standard is a useful tool that when utilized correctly can increase the precision of the research by accounting for any error outside of the researchers hands. A good example of such error would be daily minor fluctuations in the GC/MS. The validity of using an internal standard (INST) with these compounds was tested by adding the internal standard to obtain a 25 µg/mL concentration in the mixture. After analyzing the sample, two different methods of calculating a calibration curve were used. First a calibration curve was made plotting abundances versus concentrations, and the R^2 value was calculated. Next, a calibration curve

was made by plotting the (abundance of the compound/abundance of INST) versus the concentration. Figures 10-17 are representative calibration curves with Figures 10-13 being nitromethanes and Figures 14-17 being acetonitriles. The curves are also representative by showing several different halogenation configurations. Table 5 is a comparison of the INST R^2 values that clearly shows that a more precise calibration curve can be obtained when using the INST to perform the calculations. This data demonstrates that the use of an internal standard is a viable and useful tool to use with these compounds and should be used when testing and calculating unknown samples.

Table 5: Comparison of INST R^2 Values

Compound	R^2 without INST	R^2 with INST
Dibromonitromethane	0.9352	0.9954
Dibromochloronitromethane	0.9383	0.9725
Iodoacetonitrile	0.8845	0.9812
Trichloroacetonitrile	0.9111	0.9699

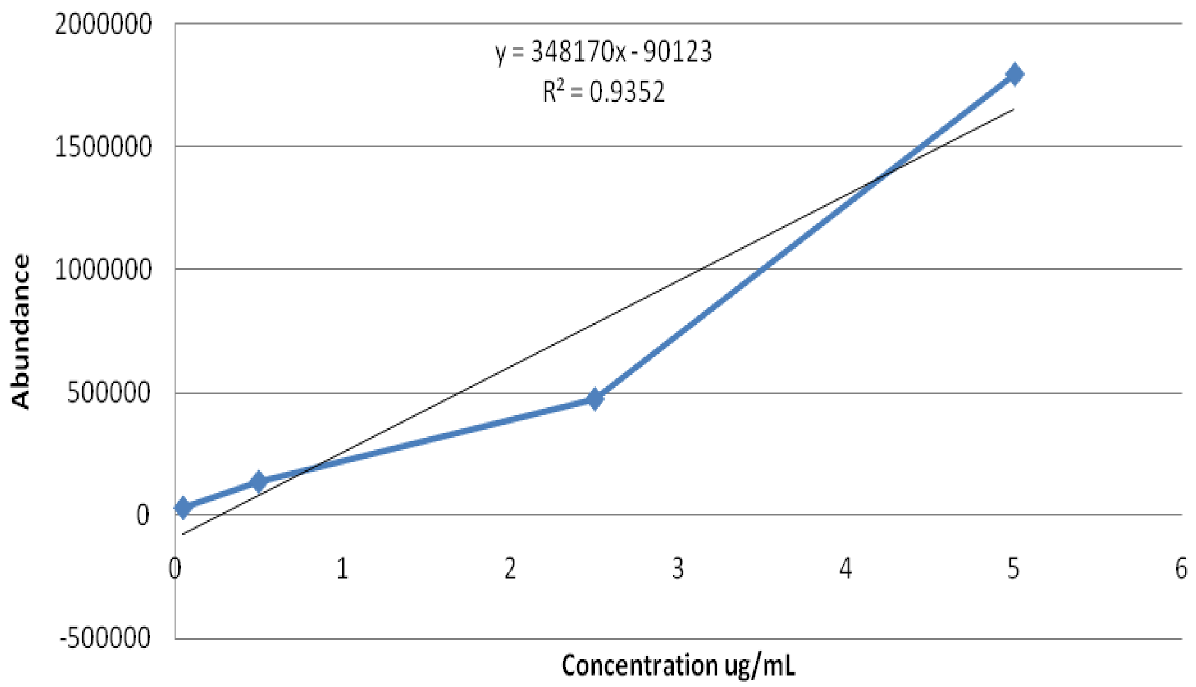


Figure #10: Dibromonitromethane without INST
y = 348170x - 90123
R² = 0.9352
Abundance
2000000
1500000
1000000
500000
0
-500000
0
1
2
3
4
5
6
Concentration ug/mL

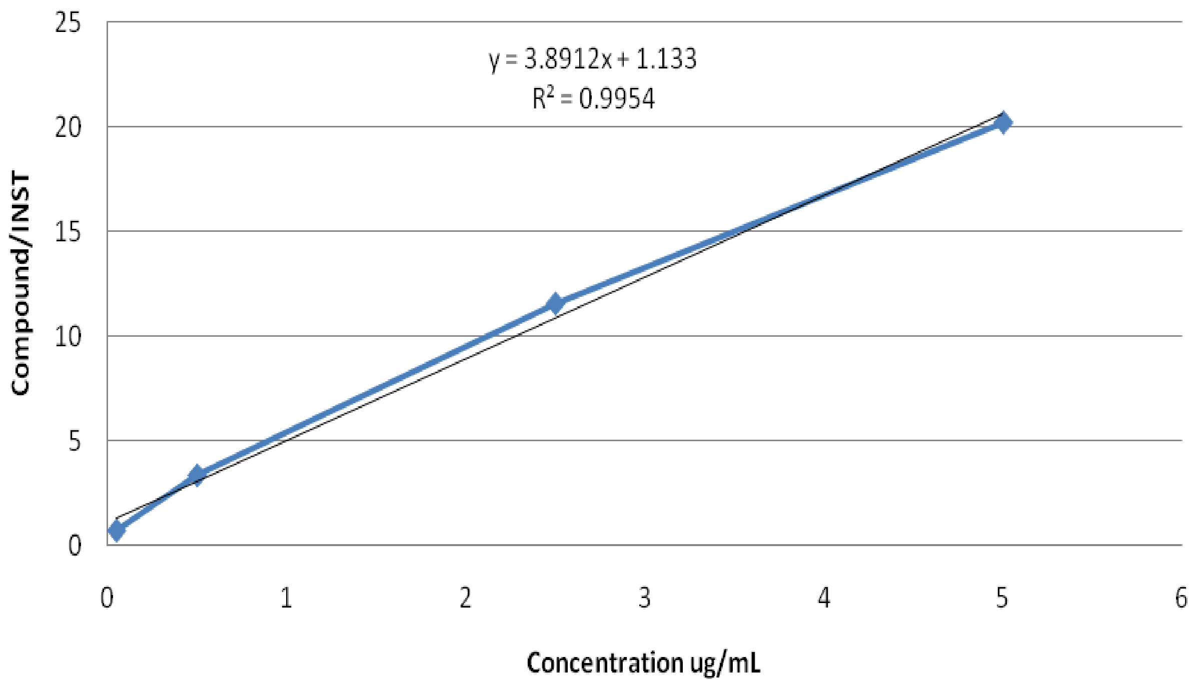


Figure #11: Dibromonitromethane with INST
y = 3.8912x + 1.133
R² = 0.9954
Compound/INST
25
20
15
10
5
0
0
1
2
3
4
5
6
Concentration ug/mL

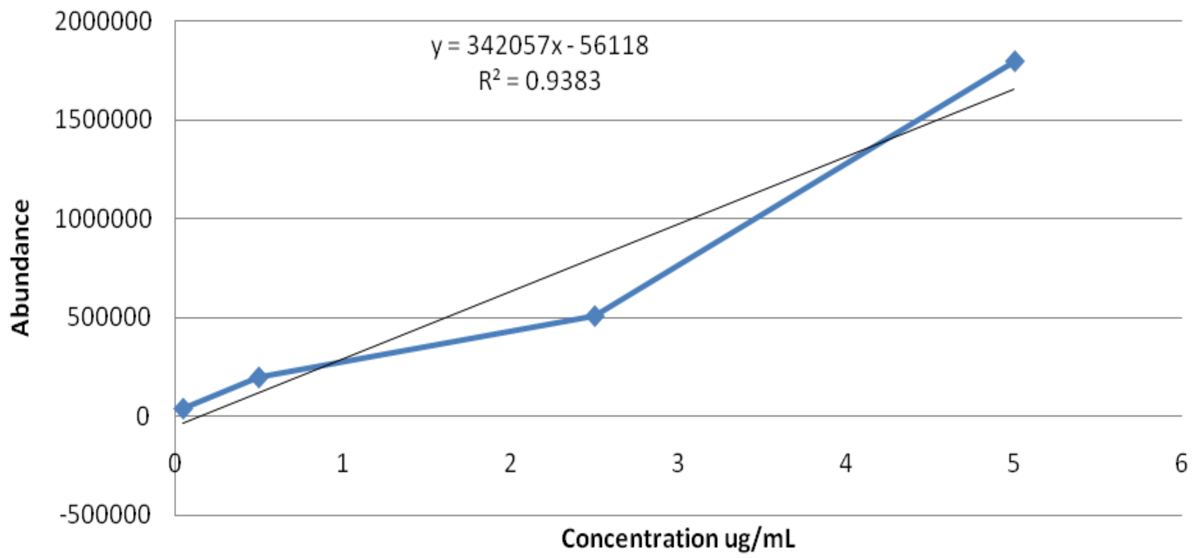


Figure #12: Dibromochloronitromethane without INST
y = 342057x - 56118
R² = 0.9383
Abundance
2000000
1500000
1000000
500000
0
-500000
0
1
2
3
4
5
6
Concentration ug/mL

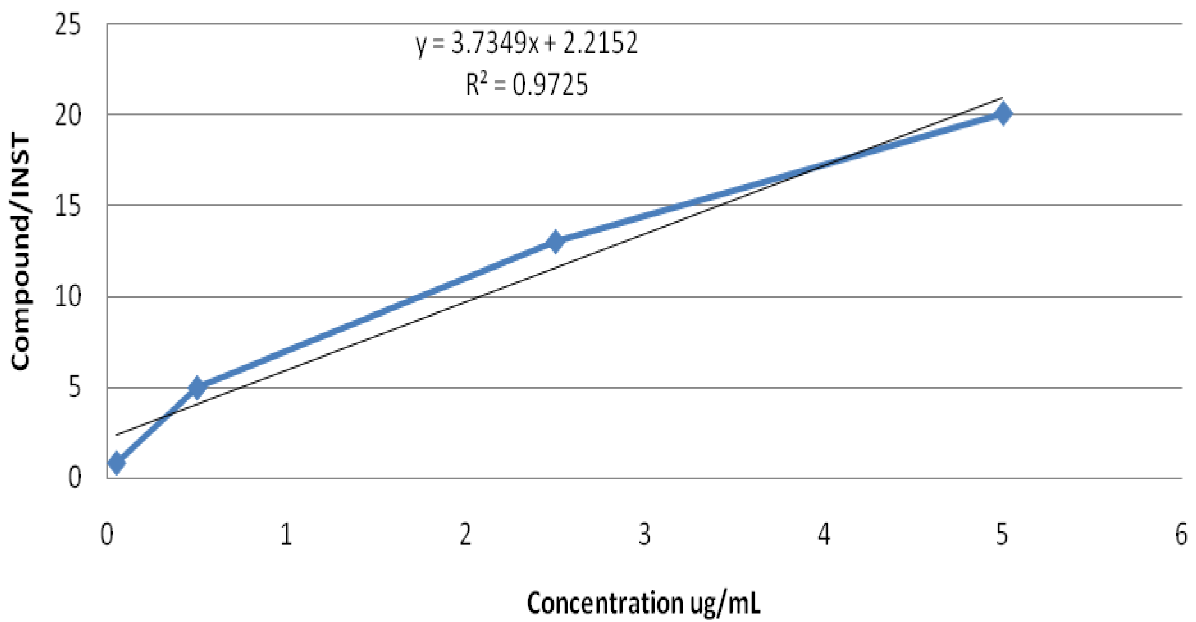


Figure #13: Dibromochloronitromethane with INST
y = 3.7349x + 2.2152
R² = 0.9725
Compound/INST
25
20
15
10
5
0
0
1
2
3
4
5
6
Concentration ug/mL

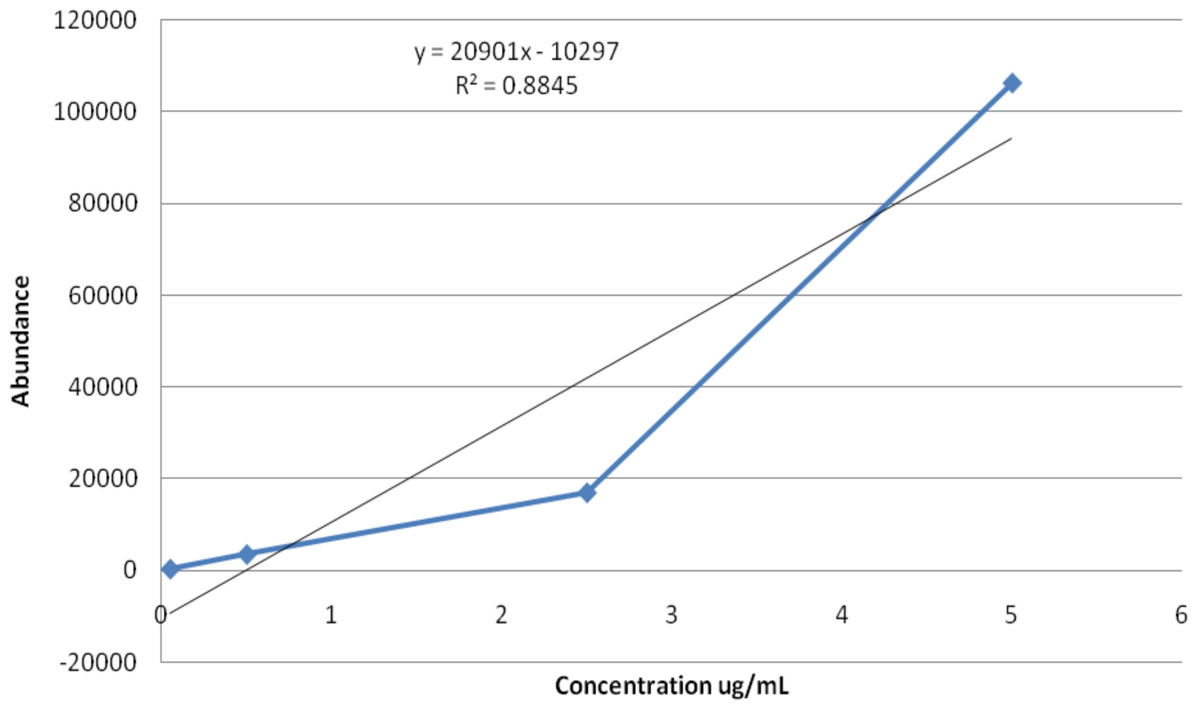


Figure #14: Iodoacetonitrile without INST
y = 20901x - 10297
R² = 0.8845
120000
100000
80000
60000
40000
20000
0
-20000
Abundance
0
1
2
3
4
5
6
Concentration ug/mL

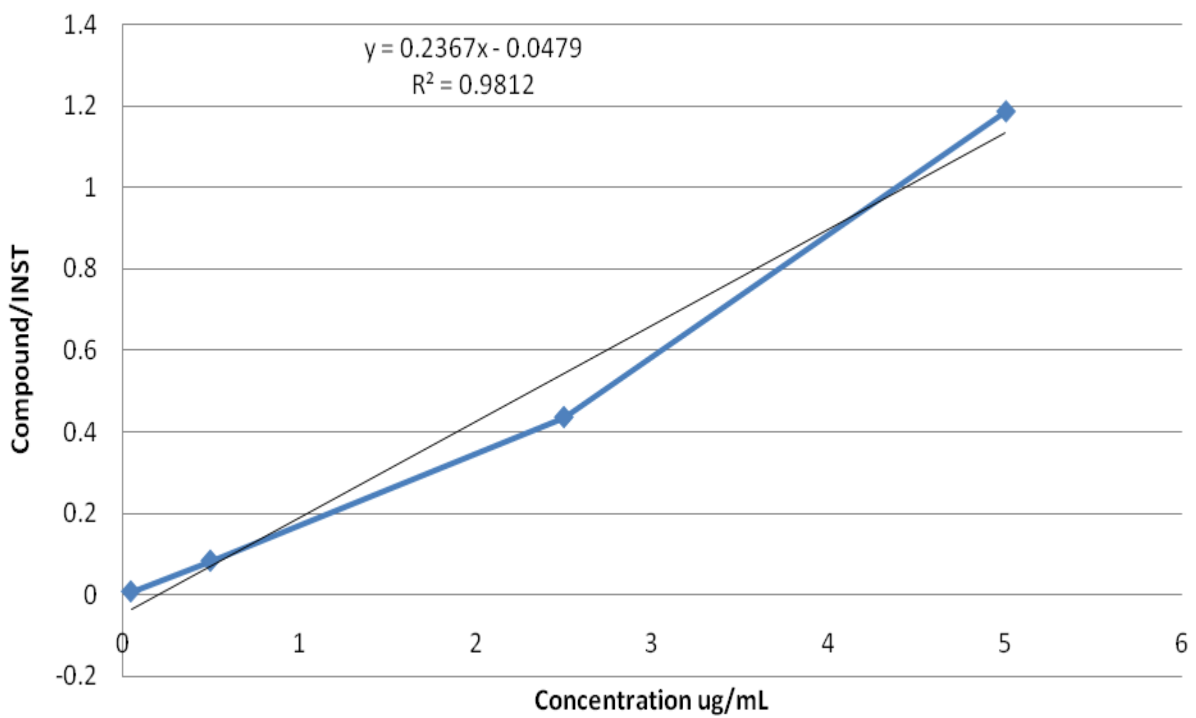


Figure # 15: Iodoacetonitrile with INST
y = 0.2367x - 0.0479
R² = 0.9812
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
Compound/INST
0
1
2
3
4
5
6
Concentration ug/mL

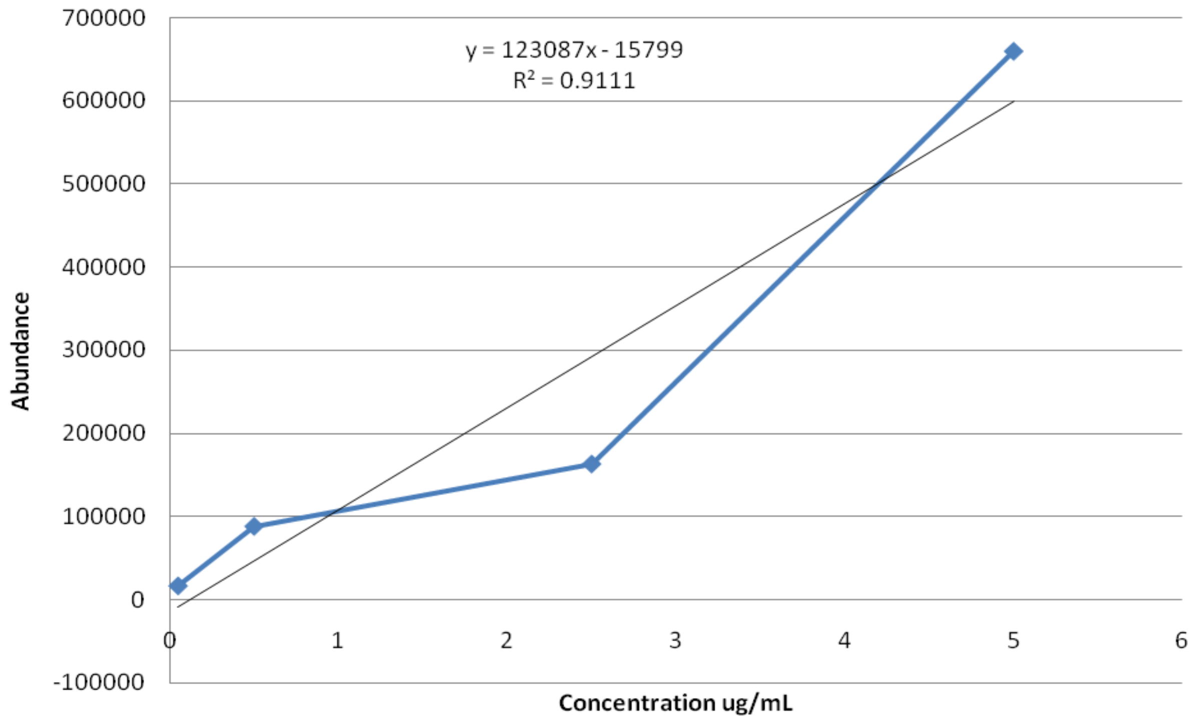


Figure #16: Trichloroacetonitrile without INST
y = 123087x - 15799
R² = 0.9111
Abundance
700000
600000
500000
400000
300000
200000
100000
0
-100000
0
1
2
3
4
5
6
Concentration ug/mL

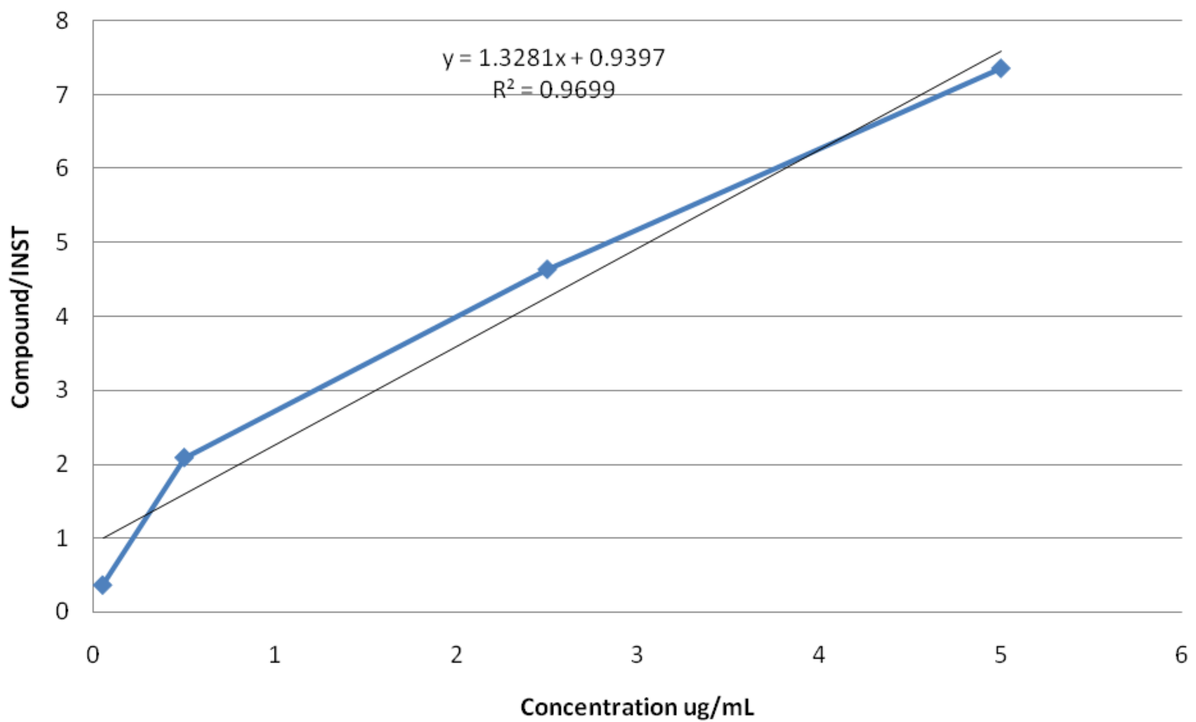


Figure #17: Trichloroacetonitrile with INST
y = 1.3281x + 0.9397
R² = 0.9699
Compound/INST
8
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
Concentration ug/mL

CONCLUSION

The aim of this research project was to focus on N-DBPs, specifically haloacetonitriles, halonitromethanes, and haloacetamides, to determine the optimal extraction parameters using SPME. From the research conducted, it was determined that neither the 60 μm Carboxen/PDMS nor the 65 μm PDMS/DVB fiber was suitable for extracting haloacetamides. From the first extraction in Figure 1 to the last extraction in Figure 5 after optimizing all of the SPME parameters, it can be observed that the abundance of most compounds was significantly higher in the optimized method than in the initial method of extraction. Thus, it has been concluded that the optimal SPME extraction parameters for this class of compounds as a whole are an extraction time of 60 minutes, a stir rate of 250 rpm, a temperature of 25°C, and fiber type of PDMS/DVB.

FURTHER STUDY

Although this study answered many questions about the nature of N-DBPs and SPME, there were still many unresolved questions that could be cause for further study. It is peculiar that the halonitromethanes and the haloacetonitriles both behaved in similar fashions when dealing with SPME, yet the haloacetamides reacted differently to the SPME fibers. It would be cause for further study to find the optimal fiber for extraction of haloacetamides and then determine the optimal conditions for extraction using that fiber.

For a more accurate representation of these N-DBPs and their true optimal method of extraction, a compound by compound analysis should be completed that tests each of the parameters tested here to make an optimal method for each individual compound. The ease of finding a method for the family as a whole simplifies the process immensely, but does not necessarily produce the optimal extraction for each individual compound as is evident from the parameters not producing positive results in all compounds.

There should be a study conducted to determine if any of these analytes are partitioning in the headspace. The study should be done by SPME analysis in the headspace of the sample to determine if headspace or immersion SPME is a more effective or more precise method of analyzing NDBPs.

Lastly, a correct holding time study should be conducted. The holding time study conducted for this project did not produce usable data. The study was to use an internal standard and was not properly utilized. Due to time constraints another study could not be conducted in time to complete the study.

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