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14. ABSTRACT

In the event of a toxic chemical release, such as an incident involving chemical warfare agent compounds, rapid and accurate response is needed to mitigate harm to the public and to emergency responders. The ability to perform rapid air sampling and subsequent fast separation and analysis of the chemical compounds using a fieldable laboratory-quality analytical instrument in order to properly characterize the chemical threat would provide an increased level of awareness and safety. This research discusses the results of applying a newly developed dynamic air sampling technique, known as High-Surface Area Solid Phase Micro Extraction (HSA-SPME), in the sampling and analysis of chemical warfare agent surrogate and degradation compounds. Use of this device at high sample flow rates (4 L?min-1) exhibits increased extraction efficiency in terms of mass per unit time relative to traditionally used emergency response detection methods, which is beneficial for decision making and minimizing time in a contaminated environment in a field response scenario when time is of the essence. Use of a tandem HSA-SPME method, in which two devices are joined in series and the sorbent surface area is doubled, permits low detection limits (<1 ppbv by GC/MS) with short sampling times (10-15 s). The speed, sensitivity and ease-of-use are demonstrated in comparison with a laboratory accepted standard chemical pre-concentration module.

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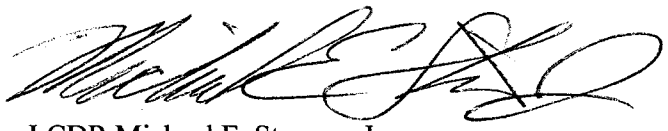

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September 30, 2009

ABSTRACT

"IMPACT OF USING A HIGH SURFACE AREA SOLID PHASE MICROEXTRACTION DEVICE AND FAST GAS CHROMATOGRAPHY HEATING RATES IN THE SAMPLING AND ANALYSIS OF TRACE LEVEL CHEMICAL WARFARE AGENTS AND CWA-LIKE COMPOUNDS"

by

LCDR Michael E. Stevens, Jr., Doctor of Philosophy, 2009

Dissertation Directed by: CDR Philip A. Smith
Associate Professor
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In the event of a toxic chemical release, such as an incident involving chemical warfare agent compounds, rapid and accurate response is needed to mitigate harm to the public and to emergency responders. The ability to perform rapid air sampling and subsequent fast separation and analysis of the chemical compounds using a fieldable, laboratory-quality analytical instrument in order to properly characterize the chemical threat would provide an increased level of awareness and safety. This research discusses the results of applying a newly developed dynamic air sampling technique, known as High-Surface Area Solid Phase Micro Extraction (HSA-SPME), in the sampling and analysis of chemical warfare agent surrogate and degradation compounds. Use of this device at high sample flow rates (4 $L \cdot min^{-1}$) exhibits increased extraction efficiency in terms of mass per unit time relative to traditionally used emergency response detection methods, which is beneficial for decision making and minimizing time in a contaminated environment in a field response scenario when time is of the essence. Use of a tandem

HSA-SPME method, in which two devices are joined in series and the sorbent surface area is doubled, permits low detection limits (<1 ppb_v by GC/MS) with short sampling times (10-15 s). The speed, sensitivity and ease-of-use are demonstrated in comparison with a laboratory accepted standard chemical pre-concentration module.

While rapid sampling is an important first step in providing answers to the incident commander or officer in charge at the scene, the advantage of decreased sampling time would be negated if the amount of time required for analysis were not optimized as well. For this reason, the impact on the retention index (RI) values when using rapid temperature programming gas chromatography rates relative to a rate of 10 $°C \cdot min^{-1}$ when analyzing 5 chemical warfare agents and one chemical warfare agent degradation product was examined. Pronounced shifts in RI values with respect to *n*-hydrocarbon reference standards were observed for several of the compounds analyzed using temperature ramp rates as high as 120 $°C \cdot min^{-1}$. This was explained by considering the increase in time-weighted average column temperature between injection and elution at higher temperature programming rates, and comparison to retention factor (k) values obtained from isothermal analyses of the same compounds. The impact of fast temperature programming rates on RI values was reproducible, and linear temperature program retention characteristics (k'_{LPTGC}) versus time-weighted average column temperature from time of initial column migration to time of elution were closely related to k versus isothermal analysis temperature.

“IMPACT OF USING A HIGH SURFACE AREA SOLID PHASE MICROEXTRACTION DEVICE AND FAST GAS CHROMATOGRAPHY HEATING RATES IN THE SAMPLING AND ANALYSIS OF TRACE LEVEL CHEMICAL WARFARE AGENTS AND CWA-LIKE COMPOUNDS”

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A dissertation submitted to the Faculty of the

Department of Preventive Medicine and Biometrics,

Uniformed Services University of the Health Sciences

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of

DOCTOR OF PHILOSOPHY
IN ENVIRONMENTAL HEALTH SCIENCES

DEDICATION

I dedicate this work to God, the Creator of all that is seen and unseen, as all glory and honor are Yours, Father. John 3:16

To my Wife, Kathleen…where do I begin? You are my Love, my Life, my Best Friend, and I could never imagine going through life without you. Thank you for your undying support and love throughout this time and throughout our marriage. You have made me so happy.

To our three amazing children, Luke, Sophia and Jack. How you fulfill my life and fill me with such inner joy. Thanks for helping 'Daddy' see life through the eyes of a child again.

To Mom and Dad, you have been my bedrock and my biggest fans throughout my life. You instilled in me a deep desire to continue learning and to face all of life's obstacles with faith, courage and strength. The only way that I could ever repay your love and support is to provide my own children with a home filled with love, happiness and laughter.

To my brothers, Jim and Ryan. Brothers like you are worth more than all the riches in the world.

To my grandparents, Gene and Louise Limp…unquestionably the grandest of all grandparents!! Your example of enduring love for one another and for your children and grandchildren is truly beautiful.

I would also like to dedicate this work to the many family, friends and acquaintances not mentioned here who have influenced my life in a positive way and have encouraged and believed in me through all the stages of my life. I am truly blessed to have so many wonderful people in my life. There is no way to list you all in a single dedication, but may God bless you all.

To the enduring memory of my cousin Lance Corporal Kevin (Adam) Lucas, 3rd Battalion, 8th Marines, 2nd Marine Division, 2nd Marine Expeditionary Force, who gave all in Anbar province, Iraq, on May 26, 2006.

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Chapter 1

Introduction

Significant health effects such as blistering of the skin, damage to the eyes, mucous membranes and internal organs, as well as respiratory injury and even death have been associated with brief human exposures (measured in minutes) to specific toxic industrial compounds (TICs) and chemical warfare agents (CWAs) as low as parts per billion by volume (ppb_v) and even parts per trillion by volume (ppt_v) concentrations [1]. Failure to detect the presence of CWAs could lead to substantial toxic exposure for large numbers of people, both at the scene of the accidental or intentional release and at the critical care facilities to which casualties are taken. While current field analytical techniques such as length-of-stain tubes, photoionization detectors (PIDs) and ion mobility spectrometry (IMS) can provide a detection response in a matter of a few seconds or minutes, these tools are not considered definitive and may not be sensitive enough to detect at levels which may cause illness or death.

While definitive analysis is desired in high-risk situations involving quantitative air sampling of a toxic chemical release, such analysis historically has required movement of a large air sample volume through a sorbent bed to ensure that a sufficient mass of the chemical is captured and delivered into an analytical system. Since air sampling for volatile organic compounds (VOCs) using traditional air sampling techniques is generally operated at flow rates of 0.2 liters per minute or less, the process of sampling a large air volume typically equates to a long collection time [2]. Answering the questions “can we collect enough mass of a highly dangerous chemical or related compound in only a matter of seconds to provide for definitive detection at ppb_v and ppt_v

concentrations?" and "can we provide predictable, definitive, on-site analytical results for CWAs even when performing the separation and subsequent analysis of these compounds in significantly faster time relative to standard operating conditions?" comprised the main focus of this research. The two major focal points were: 1) the examination of a high-volume, high flow rate air sampling method known as high surface area solid phase microextraction (HSA-SPME), as it is applied in the analysis of chemical warfare agent surrogate and degradation compounds, and 2) the impact of using fast temperature gas chromatography (GC) programming in the analysis of chemical warfare agents and a chemical warfare agent degradation compound.

A near-ideal sampling and analysis method would possess several features. Sampling would be performed in seconds. The analytical method would include the capability to adequately separate and distinguish between different compounds, while subsequently detecting each specific compound at trace concentration levels (ppb_v and ppt_v) with near real-time analytical response. Such a method would permit the field operator to rapidly and conclusively answer the question of what compounds exist in the sample, providing for greater health protection and safe zone integrity.

In addition to providing an increased safety factor, improved field sampling and analysis methods afford the ability to quickly communicate the level of risk posed to the public from a chemical agent release. While often an overlooked aspect, timely and effective risk communication is critical in averting widespread panic. A situation in which hospital assets are inundated with the "worried well," those people who have not actually been exposed to a harmful chemical but are concerned that they may have been, can quickly become a major hindrance in caring for those who are actually exposed. In

1995, a deliberate chemical warfare agent (sarin) release in a Tokyo subway killed 12, but caused local hospitals to be overwhelmed with over 5,500 people, the majority of which required no medical treatment [3]. An on-site, rapid sampling and analysis method, capable of providing detection at levels below those harmful to human health, may help alleviate the feelings of fear and panic. This can result from the lack of human health risk information in the aftermath of the disaster, by providing near-immediate communication of relative safety for the majority of the public.

Additionally, a rapid means of sampling VOCs would also be important for the forensic science community. Criminal investigations involving the detection of human scent profiles for criminal suspects or scent emanating from clandestine gravesites containing human remains would be aided by the use of a selective and rapid chemical sampling method. Detection of the unique chemical signatures from drugs and explosives would also be enhanced by employing such a sampling method. Historically, canines have been trained by law enforcement units in order to identify and alert to certain trace level volatiles, while disregarding other unrelated trace compounds [4]. A portable vacuum, known as the Scent Transfer Unit^{TM} (STU-100), is used to capture and concentrate the volatile organic signature emissions from an evidentiary item of interest. The organic volatiles are collected on cotton gauze and a trained canine is then allowed to sniff the scent contained on the gauze. A handler then follows the dog as it attempts to identify the source of the volatile organic signature, either in a criminal suspect line-up or at various suspected crime scenes [5-7].

While the canine's ability for nearly instantaneous detection of specific scent profiles at very low concentrations has been demonstrated in the capture of fugitives,

location of missing persons, and in the detection of buried human remains, this method has not been fully characterized and is not completely understood. Validation in terms of chemical compounds detected, the relative ratios of the different compounds, as well as detectable quantities, cannot be determined simply by observing canine response [8]. Harvey and Harvey underscored an additional concern regarding canine evidence reliability when attempting to match an evidentiary item with the sought-after suspect or victim. Their findings indicated that the success rate of novice bloodhound and handler teams was only 53 percent, compared to 96 percent with more experienced teams [9], indicating that the canine method requires a large investment of training time to be effective. Pursuing potentially misleading signals can result in hours of wasted time, while providing little useful information towards solving the criminal case. Also, the use of a canine would not be appropriate in attempting to identify concentrated hazardous chemicals due to obvious health hazards posed to the animal [4].

Solid phase microextraction (SPME) has been successfully utilized in the non-exhaustive extraction of volatile and semi-volatile organic compounds for nearly 2 decades. The commercially-available SPME extraction device consists of a fused silica fiber which is coated with a liquid or a combined liquid-solid sorbent film. The SPME fiber is typically used to passively extract chemical compounds from the air upon exposure to a contaminated atmosphere, based on equilibrium constants between the atmosphere and coating [10]. However, the use of a passive equilibration-based air sampling method for quantitative collection of semi-volatile compounds may take several hours or more for some chemical compounds.

Dynamic, non-equilibrium-based SPME methods have been investigated previously to determine if they improve the rate of mass uptake into/onto the SPME sorbent for compounds of interest. Previous studies using a high volumetric flow (4 $L \cdot min^{-1}$) have indicated that the faster flow rate relative to traditional air sampling methods and relative to passive SPME methods, allows for greater mass uptake of the chemical of interest when a fixed sample time is used [11-14]. If a similar or greater amount of airborne chemicals can be collected from a potentially contaminated environment in a fraction of the time, using faster flow rates relative to hours-long low flow traditional methods, the high surface area SPME (HSA-SPME) method discussed in these references could represent a more effective means of sample collection when time and safety are of the essence.

In this study, examination of HSA-SPME involves determining the sensitivity of the method when operating the device for different fixed sampling times and different sampling volumes in the analysis of trace concentrations of a CWA surrogate compound. The HSA-SPME method chemical sampling results are compared to results from using a commercially available pre-concentrator with a sorbent-filled sample tube and focusing trap. Sampled airflow of gas phase chemical compounds across the sorbent bed of the pre-concentrator sample tube is a similar means of collection to the EPA-accepted method of VOC sampling [15], and represents the control for the study. Additionally, the results of sampling and subsequent analysis of a gas-phase chemical warfare agent surrogate and a liquid phase chemical warfare agent degradation product when using a tandem HSA-SPME method are reported. The tandem HSA-SPME sampling method consists of two HSA-SPME devices connected in series.

While decreasing the sampling time necessary to collect an adequate amount of analyte mass for detection would represent an improvement in field operations, this advantage would be negated if the amount of time required for analysis were not optimized as well. While the standard laboratory methods of analysis for trace level volatile and semi-volatile organic compounds has included GC/MS, the traditional GC/MS system is not well-suited for fast separation and analysis. The term 'fast separation' in this context is defined as separation of an injected chemical mixture when using GC temperature heating rates greater than 10 $^{o}C \cdot min^{-1}$ [16].

The advent of resistively heated fast GC instrumentation approximately 10 years ago permits rapid column heating [17]. If retention data obtained under typical GC conditions are to be used with rapid GC column heating and rapid separation, it is important to understand how deviation from the standard ramping conditions of 10 $^{o}C \cdot min^{-1}$ used to obtain published retention data (specifically retention index values, or RIs) will impact their usefulness [16]. If under the conditions needed for rapid separations, compounds of interest exhibit non-proportional changes in retention relative to reference hydrocarbons, RI data would still be of benefit as long as deviations from standard retention behavior are predictable and reproducible.

When investigating the effect of non-standard GC temperature ramping rates on retention data for five chemical warfare agents and one CWA degradation product in this work, both isothermal and linear programmed temperature GC heating rates were used. The impact of using higher isothermal temperatures and faster linear temperature heating profiles on the retention behavior of these compounds, specifically retention indices, was characterized and reported. Additionally, the impact of using a range of carrier gas linear

velocities and different carrier gases on the retention indices of these compounds was also examined.

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Chapter 2

Literature Review

2.1 Solid Phase Microextraction

Developed by Pawliszyn in 1990 as a passive sample collection method for volatile and semi-volatile organic compounds, SPME is a re-usable, lightweight, easy-to-use sampling technique [1,2]. The SPME sampling method has been used successfully in the field for nearly 20 years in the collection of VOCs and semi-volatiles, to include toxic compounds such as CWAs [3-5]. The polymer coating of the SPME device may act as both an absorbent and adsorbent, depending on its specific coating components, in partitioning chemicals into the bulk polymer or onto its surface from the surrounding environment. The SPME method is capable of collecting and concentrating most analytes suitable for gas chromatography (GC) analysis and is effective over a large range of analyte concentrations. For absorptive sampling of air, the partitioning of the chemical between the two phases (sampled environment and the SPME polymer coating) is represented by the distribution constant, K [1]. This distribution constant is described mathematically in Equation 2.1.

(2.1) $$K_{fg} = C_f / C_{air}$$

The notation K_{fg} represents the distribution constant between the SPME fiber coating and the surrounding atmosphere, the notation C_f represents the concentration of the particular chemical compound in the polymeric fiber coating, while C_{air} is the concentration of the compound in the surrounding air. Many different SPME polymer coating types and

thicknesses are commercially available, and selection of a particular material is according to the coating's similarities to the chemical and physical properties of the analytes to be collected.

When used in air sampling, chemical components begin to immediately transport onto or into the SPME coating [1]. For absorptive coatings under equilibrium conditions, the amount of analyte mass extracted corresponds directly to analyte concentrations in the sampled matrix as defined by Equation 2.1 and the fixed volume of the SPME fiber coating. No solvent extraction is required in the preparation of SPME samples for GC analysis, thus eliminating a time-consuming step in the analytical process while providing for greater detection sensitivity due to the lack of both solvent background and sample dilution.

2.1.1 Dynamic Solid Phase Microextraction

While providing a selective, sensitive and more portable means for collection and subsequent identification for trace level organic volatiles and semi-volatiles in the field for quantitative detection, equilibrium-based SPME sampling can be time-consuming and not as sensitive as more traditional non-equilibrium environmental sampling methods [6]. Achieving equilibrium between the SPME fiber and the vapor headspace above the chemical is not always feasible where rapid collection and analysis are necessary. Previous research has indicated difficulty in equilibrating VOCs and semi-volatile compounds with retention index (RI) values greater than 1300 into a non-polar polydimethylsiloxane (PDMS) phase coating, despite sampling durations of 30 minutes at 25 ^{o}C [6]. A non-equilibrium sampling method which is effective in reducing the time

required to collect and subsequently detect and potentially quantify both volatile and less volatile compounds of interest would represent an improvement from equilibrium-based SPME methods.

In order to determine if a more time-efficient active SPME method could be developed, several studies have previously been conducted. Koziel *et al.* developed a means of delivering an air stream at a perpendicular angle to a SPME fiber for a fraction of the time that would be needed for static equilibration. This method resulted in a significant increase in the mass of analyte material diffusing onto the fiber coating per unit time [7]. Augusto *et al.* built on this dynamic SPME work, developing a field-portable dynamic air sampling (PDAS) device, designed for collection of trace volatile organics. This consisted of a SPME fiber coated with adsorptive polymer, contained within a metal housing with a perpendicular airstream inlet [8]. During sampling, airflow through the device produced a constant, uniform air flow around the SPME fiber. It was determined that the mass uptake of sampled volatile organics into the fiber became essentially constant at an air speed above 10 $cm \cdot s^{-1}$ linear velocity.

Results using the PDAS-SPME device indicated an increase in the number of compounds detected and sensitivity, while also reducing sampling time by several orders of magnitude when compared to National Institute of Occupational Safety and Health (NIOSH) air sampling methods. Additionally, a greater amount of analyte mass was adsorbed by the fiber coating in comparison with traditional SPME methods [8]. Hook *et al.* later demonstrated the utility of a PDAS-SPME device in the identification and quantification of sarin. The dynamic method detected and quantified sarin at 0.10 $mg \cdot m^{-3}$,

half the concentration level considered immediately dangerous to life and health, after only a 30-second sampling time with a PDAS-SPME device [9].

Bartelt *et al*. developed an active sampling technique for use in the laboratory in which a SPME fiber coated with absorptive PDMS was placed inside a custom-made laboratory-based air sampling apparatus. The apparatus was designed to produce air flow in a parallel direction with the fiber [6,10]. This dynamic sampling method was intended to reduce the time required to collect and quantitate chemical compounds which would normally take several hours or days to equilibrate with equilibrium-based SPME methods. This dynamic SPME method significantly reduced the time required to produce a quantitative response for semi-volatile compounds compared to using equilibrium-based SPME methods.

While these dynamic SPME methods represented significant steps forward in reducing the amount of sampling time required to extract adequate compound mass for subsequent detection, they are either not considered field techniques or are not as user friendly in the field as desired. Most use fragile glassware or require controlled laboratory conditions, which preclude use in the field. Researchers at the Counter Terrorism and Forensic Science Research Unit (CFSRU) of the U.S. Federal Bureau of Investigation Academy (Quantico, VA) postulated that a fieldable, easy to use air sampling device could be developed, based on a dynamic SPME technique. Development and initial testing of a novel high surface area SPME (HSA-SPME) air sampling device was accomplished in 2004 [11].

2.1.2 High Surface Area Solid Phase Microextraction

The research of Ramsey [11,12] involved coupling the HSA-SPME sampling device to a traditional laboratory-based gas chromatography/mass spectrometry (GC/MS) system in the sampling and analysis of a standardized volatile organic compound preparation. The GC/MS system was equipped with a focusing pre-concentrator containing a dual carbon-based adsorbent bed. Use of this method provided an improvement of 1-2 orders of magnitude in detection of an EPA Compendium TO-14 mixture (39 volatile organic compounds) when compared to static SPME/GC/MS and the dynamic, perpendicular flow rate PDAS-SPME/GC/MS methods [11,12].

The HSA-SPME element consists of a 10-centimeter length nickel alloy wire coated with 65 μm thick adsorbent/absorbent SPME stationary phase, contained between two concentric glass tubes. The nickel alloy wire can be coiled in different spiral configurations ranging from a tight spiral (1.1 mm between coils) to a loose spiral (4.4 mm between coils) between the two concentric glass tubes. The alloy chosen as the substrate for the SPME polymer is designed for oxidation resistance and is capable of the large temperature desorption range required (room temperature to 300^{o} C) for efficiently removing sampled volatile and semi-volatile organic compounds from the polymer surface of the collection device [11,12].

Carboxen-PDMS was the stationary phase applied to the HSA-SPME nickel alloy wire. This coating was chosen for its selectivity for a wide range of VOCs and its established durability when sampling at relatively high flow rates [7]. MacDonald confirmed Carboxen-PDMS was a more stable polymer coating for use with high flow sampling than PDMS coating alone which rapidly degraded and was stripped from the

nickel alloy wire at flow rates of 1.5 $L \cdot min^{-1}$ and higher [13]. Additionally, the large surface area of Carboxen 1000 (>800 $m^2 \cdot g^{-1}$), makes it an excellent VOC adsorbent [14]. This sorbent material is designed for collection of lighter and more volatile compounds due to the size and high surface area of its pores. Despite its affinity for smaller non-polar compounds, Carboxen 1000 also exhibits good desorption efficiency (release of the collected compounds) upon heating, which can occur at temperatures up to 400 ^oC without sorbent degradation. Also, while Carboxen-PDMS can be subject to competitive sorption effects, these were minimized in previous research with the use of short sampling times, as is the case with the dynamic HSA-SPME method [15].

The geometry of the HSA-SPME device creates an annular space for airflow between the coating and the outer tube and also results in a polymer surface area which is ten times that of a commercial SPME device. A calculated surface area (mm^2) to vapor volume (μL) ratio of 1:2.8 [11,12] is achieved when using the 10-cm nickel alloy wire length. Using the HSA-SPME method, active collection is performed by drawing a volume of sampled air rapidly over the HSA-SPME element using a small, battery-powered pump connected with plastic tubing to the back end of the element. Desorption of the polymer-coated wire is performed by resistively heating the element using electrical current.

Previous research results suggest that the relatively high surface area to vapor volume ratio of the HSA-SPME device allows for greater opportunity of analyte-polymer contact, giving greater extraction efficiency of analyte mass per unit time when compared to static SPME methods [11,12]. Therefore, improved detection sensitivity may result while sampling for shorter durations. An important goal in the quantitative sampling and

analysis of toxic airborne compounds is to rapidly extract enough analyte mass from the contaminated atmosphere to provide for subsequent detection. The HSA-SPME approach represents a potentially useful field sampling method to help reduce personnel exposure time in contaminated environments by decreasing the time needed for this.

Additional HSA-SPME research has investigated optimum sampling flow rates, the impact of high flow rates and high-temperature desorption on the phase coating, and the effect of different geometries on the device's extraction efficiencies when coupled with traditional, bench top GC/MS analytical instrumentation equipped with a cryogen-based pre-concentrator system [13,16]. MacDonald evaluated the extraction efficiency of the HSA-SPME sampling device at six different sampling flow rates, ranging from 0.1 $L \cdot min^{-1}$ to 10 $L \cdot min^{-1}$, and compared the total compound extraction over this range. Results of sampling a 10 ppb_v concentration of an EPA Compendium TO-14 mixture of 39 volatile organic compounds indicated that greater extraction efficiency relative to 10 $L \cdot min^{-1}$ flow rate was observed at 0.1 $L \cdot min^{-1}$ flow rate when sampling a fixed volume. For fixed sampling times, however, the 10 $L \cdot min^{-1}$ flow rate far exceeded the lower flows in terms of mass collected per unit time, based on significantly higher analytical response for the 10 $L \cdot min^{-1}$ flow rate method [13].

Due to its helical design, it was initially hypothesized that the increased extraction efficiency of HSA-SPME over other passive and active SPME methods may be influenced not only by its increased surface area, but also to some degree by its geometry. Wheeler examined five different HSA-SPME geometries in attempting to determine the effect of the geometry of the HSA-SPME device's phase-coated wire on extraction efficiency. The surface area of each of the devices was the same, but the geometries of

each coated wire ranged from a tightly wound helical coil to a straight wire design. The results indicated that no significant difference existed between the analyte uptake of any of the different geometries at 1 $L \cdot min^{-1}$ and 4 $L \cdot min^{-1}$ flow rates [16].

2.2 GC/MS Analysis

Consisting of two distinct and different technologies, gas chromatography/mass spectrometry (GC/MS) is capable of providing definitive analysis for a wide range of organic compounds. Sensitivity using this dual technology can approach 0.1 nanograms in full scan mode [17]. As this research focused in part on the rapid separation of unknown chemical mixtures in the field, the "front end" component of GC/MS, or gas chromatography, will be briefly described and discussed.

Gas chromatography, first introduced by James and Martin in 1952 [18], can act as a partition-based or adsorption-based technique. In general, currently used GC involves introduced chemical compounds moving between the stationary phase and the gaseous mobile phase within a pressurized, small diameter (typically between 0.10 to 0.53 millimeter internal diameter) open tubular fused silica capillary column, which is contained within a relatively large convection oven (about 6 cubic feet). For analysis of many organic analytes, the interior surface of the column is coated with a liquid polymer film of specific polarity and thickness (measured in microns), and the rest of the inner diameter of the column is filled with inert carrier gas (the mobile phase), often helium or hydrogen.

Chemical compounds are introduced into the pressurized column at the GC inlet port. Airborne chemical contaminants are typically trapped on a sorbent bed from the

sampled atmosphere, extracted with solvent, with subsequent μL scale volume of the solvent-diluted mixture introduced into the inlet by microsyringe injection. The septum-sealed, heated inlet is maintained at a temperature high enough to vaporize the particular volatile organic mixture injected (generally 200-250 ^{o}C). After a brief time in the inlet, the vaporized sample components are carried onto the column by the movement of carrier gas from the injector into the column.

If the column is maintained at an initial temperature of 40-60 ^{o}C, introduction of vapor-state semi-volatile chemical compounds onto the much cooler column has the effect of condensing the compounds at the head of the column. The GC column is heated to facilitate analysis of compounds within a range of volatilities. Based on factors such as the initial and programmed column temperature, carrier gas flow rate, and physical/chemical interaction between compounds and the column's liquid stationary phase, the compounds will begin to migrate down the column at different rates relative to one another. This separation of the individual chemical components in the sample mixture allows identification of the separated components of the injected chemical mixture, which is the primary goal of GC [19]. The combination of GC with a mass spectrometer detector, which provides molecular identification of each of the separated compounds in the injected sample, provides sampled mixture separation and subsequent compound identification of trace level low and medium weight VOCs [20].

While GC/MS is a proven tool for VOC detection, reducing GC separation time of chemical compounds is important for time-compressed field emergent analysis. To analyze a range of compounds with a range of volatilities, a linear GC temperature profile, in which the column temperature is increased rapidly over the course of a GC

run, is often used. The retention variability caused by numerous factors can prevent reliable use of the compound retention time (t_R) parameter as an identification tool. Additionally, GC system differences can significantly impact t_R [21]. While GC/MS identification of an unknown chemical compound is frequently completed by relying only on mass spectrum matching against a digital library reference spectrum, routine use of both retention data and mass spectral match would represent an improved method for accurately identifying unknown compounds, especially in situations where significant risks could exist with misidentification of the compounds in a sample mixture.

2.3 Retention Indices

A means of adjusting for retention variability, the retention index (RI) method for isothermal temperature programs was first introduced by Kovats in 1958 [22]. The RI method compares the retention of the chemical of interest relative to benchmark chemicals, usually a series of *n*-alkanes. This chemical reference indexing technique was based on earlier findings by James and Martin, who determined that logarithms of corrected retention volumes for members of a homologous series produce a straight line when plotted against carbon number [18]. The RI method assigns an index number for each *n*-alkane by multiplying its carbon number by 100. Hence, the eight-carbon *n*-octane compound has an index of 800, the nine-carbon *n*-nonane compound has an index of 900, and so on [23].

The Kovats RI method was not intended to provide index values for compounds separated using linear temperature programming. In 1963, Van den Dool and Kratz developed a method which provides RI values based on linear temperature programming

analysis of a range of compounds with varying volatilities [24]. The Van den Dool and Kratz method has proven to be an effective means of adjusting for retention changes with GC column temperature alterations. Using this approach, the RI value is calculated for an unknown compound using the t_R values of the two *n*-alkanes eluting on each side of the unknown compound and the t_R of the unknown compound in the RI equation. The chemical's relative time position to its adjacent reference *n*-alkanes determines its RI value.

The analysis of reference compounds and comparison of t_R values has been shown to provide reproducible and reliable chromatographic identification of unknown chemical compounds when using modest linear temperature programming rates [24]. A distinct benefit of the RI system is that it permits direct comparison of inter-laboratory values. Providing this information in combination with MS analysis enhances the operator's predictive ability and provides a more complete and accurate picture of each component in an unknown sample.

The predictability of RI values when using very fast ramp rates is of interest to the field operator. Column heating rates of 2 $^{o}C \cdot sec^{-1}$ or higher are now possible with resistively heated, low thermal mass (LTM) columns. This heating capability is significantly improved from traditional GC systems, which typically do not exceed a 0.6 $^{o}C \cdot sec^{-1}$ rate. When an LTM GC column module is integrated with a mass spectrometry detector, a smaller, less power-hungry GC/MS system can be used for rapid and definitive field analysis. Originally described by Sloan *et al.*, the RVM Scientific resistively heated LTM GC column (Santa Barbara, CA) operates at only one-tenth the

power requirement of a traditional convection GC oven, while performing analyses in one-quarter of the time [25].

2.4 Resistively-heated GC Column

The RVM Scientific resistively-heated LTM GC column design used in this research couples a heater wire with a temperature-measuring component (resistance temperature detector, RTD), to a capillary column [25]. Both the heater wire and RTD are located adjacent to the capillary column throughout its length to provide resistive column heating and accurate temperature measurement. The combination of the three is coiled into a toroid configuration and completely wrapped in insulating metal foil, to isolate the thermal mass of the column during heating cycles. This configuration also allows for conductive heat exchange throughout the wound column [26]. The GC column module has been used as part of a field portable GC/MS instrument which represents one-third less size and weight relative to commercial GC/MS systems [27].

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Chapter 3

Application of a High Surface Area Solid Phase Microextraction Air Sampling Device for the Collection and Analysis of Chemical Warfare Agent Surrogate and Degradation Compounds

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Abstract

This work examines a newly developed dynamic air sampling technique, known as High-Surface Area Solid Phase Micro Extraction (HSA-SPME), in sampling and analysis of chemical warfare agent surrogate and degradation compounds. The polymer coating of this device has 10 times the surface area of commercially available SPME fibers, and at high sample flow rates (4 $L \cdot min^{-1}$) provided increased extraction efficiency in terms of mass per unit time. The high sensitivity with short sampling durations (5-60 seconds) can be beneficial for decision making and minimizing time in a field response scenario contaminated environment, when time is of the essence. Use of a tandem HSA-SPME method, in which two devices are joined in series to double the sample collection surface area (160 mm^2), permits low detection limits (<1 ppb_v by GC/MS) with short sampling times (10-15 sec). The speed, sensitivity and ease-of-use are demonstrated in comparison with a laboratory accepted standard chemical pre-concentration module used for sample introduction.

3.1 Introduction

Military and emergency response teams tasked to handle accidental or intentional releases of toxic chemicals would benefit from improvements in the speed and sensitivity of field collection techniques for chemical compounds. Typical chemical collection and analysis methods which are rapid are not considered definitive. Colorimetric (e.g. length of stain tubes) methods can lack the necessary sensitivity to provide a margin of safety for human health. Additionally, ion mobility spectrometry (IMS) methods may not provide the necessary specificity and sensitivity required [1]. Traditional industrial hygiene air sampling methods are used for precision quantitation, but sample collection can take several hours, limiting their usefulness in emergency response [2]. A sampling device capable of capturing sufficient analyte mass at trace-level detection in only seconds would represent an improvement over currently used methods.

A high speed, highly sensitive sampling device would also be of use to criminal/covert investigators. Forensics response units have historically depended on canines to detect scent (or volatiles) profiles to identify the location of buried human remains, assist in capturing a criminal suspect, or to detect the unique chemical signatures emanating from illicit drugs or explosives [3,4]. However, challenges to canine evidence regarding court admissibility have arisen due to the lack of national certification standards and insufficient scientific understanding regarding how trace human scent is detected by canines [5]. The limited number of properly trained dogs and associated costs of training and maintenance have further driven the need to look for an alternative to this chemical detection method.

High-volume sampling for short periods using a high surface area device may yield sufficient chemical mass loading for method sensitivity by increasing mass uptake per unit time. Such a sampling method would allow for a greater level of safety involving hazardous substances, as responders would spend less time in a contaminated environment. Additionally, this approach would allow for more efficient use of resources and would enable quick and discrete detection of compounds of interest in a potentially hostile environment, which would benefit military, law enforcement personnel and intelligence agencies.

A recently introduced air sampling method known as high surface area solid phase microextraction (HSA-SPME) offers the potential to provide the highly sensitive sampling capabilities that are desired by hazardous material response, law enforcement, and military personnel [6,7]. As previously described [6,7], the HSA-SPME device consists of a SPME coated wire which has a surface area ten times greater than commercially available SPME fibers, and which may use relatively high sample flow rates (4 $L \cdot min^{-1}$), sampling a large air volume in a matter of seconds. Coupling this device with a low thermal mass gas chromatograph with a mass spectrometric detector (LTM-GC/MS) for the separation and detection of volatile organic compounds allows for both rapid high-volume sampling and rapid analysis [6,7].

This research examines the use of the HSA-SPME air sampling method in the collection of trace level CWA surrogate and degradation compounds with brief sampling times. The focal points of this work include the comparison of the analytical response for a CWA surrogate compound as an airborne analyte, when sampling a 15 ppb_v concentration using the HSA-SPME method and a commercially available pre-

concentrator method. The analytical results of the methods are compared over a range of different fixed air sample volumes and different sampling times. Characterization of the limits of detection and mass uptake by use of a tandem HSA-SPME approach, in which two HSA-SPME devices are joined in series to further double the sampling surface area, is also investigated using ppb_v and ppt_v airborne analyte concentrations. Additionally, the analytical results following the rapid headspace sampling of cloth material contaminated with a small amount of a semi-volatile liquid CWA degradation compound are reported.

3.2 Materials and Methods

3.2.1. Preparation of Diisopropyl Methylphosphonate (DIMP) Air Samples

Air samples of the CWA surrogate compound diisopropyl methylphosphonate (DIMP) were generated as follows: National Institute of Standards and Technology (NIST)-traceable permeate flow of DIMP was generated by placing a permeation tube containing the chemical (HRT 010-00-5016/100^o, Kin-Tek, La Marque, TX) into a small temperature-programmed oven unit contained within the Kin-Tek model 491M-B precision gas standards generator. After allowing three days for system equilibration, the 10 cm length DIMP permeation tube produced an emission of 49 $ng \cdot min^{-1}$ (490 $ng \cdot min^{-1}$ total emission) at 100 ^oC. The diluent gas flow was adjusted to 4000 $mL \cdot min^{-1}$ to provide a concentration of 0.098 $ng \cdot ml^{-1}$ or 13 ppb_v concentration. A 10 Liter Tedlar® bag (DuPont, Wilmington, DE) was attached to the gas standards generator span outlet to collect the effluent. Lower concentrations of DIMP (3.5 ppb_v, 1.0 ppb_v, and 0.75 ppb_v) were generated by appropriate dilution of the 13 ppb_v sample using dry compressed air in separate 10 L Tedlar® bags, using a 2 L airtight syringe (Hamilton Co., Reno, NV).

3.2.2. Preparation of Headspace Sample of Liquid CWA-Degradation Product

The VX degradation compound, 2-(diisopropylaminoethyl)ethyl sulfide (2-DES) was diluted in methylene chloride to produce a 25 $ng \cdot uL^{-1}$ concentration. A 50 ng quantity of 2-DES was then volumetrically delivered onto a 1 square inch section of cotton t-shirt material, which was contained in a custom-made sampling jar with an internal volume of 250 cm^3. The solvent was permitted to evaporate for 20 min prior to sealing the sampling jar. The sealed jar was then placed in a digitally controlled heating block (Electrothermal Barnstead, Dubuque, IA) and equilibrated at 30 ^oC for 15 min prior to sampling.

3.2.3 Sampling Device

The directly heated HSA-SPME device used in this work is made up of the HSA-SPME element, glass tubing, and electrical wires. The HSA-SPME element consists of a 10 cm Stablohm 675 nickel alloy wire (60% Ni, 24% Fe, 16% Cr; California Fine Wire Company, Grover Beach, CA) coated with a 65 μ m thick Carboxen-Polydimethylsiloxane (Carboxen-PDMS) SPME stationary phase (Supelco, Bellefonte, PA). The oxidation-resistant nickel alloy wire is coiled in a loose spiral (4.4 mm between coils) around the outer surface of a small diameter borosilicate glass tube, while contained within a larger diameter glass tube.

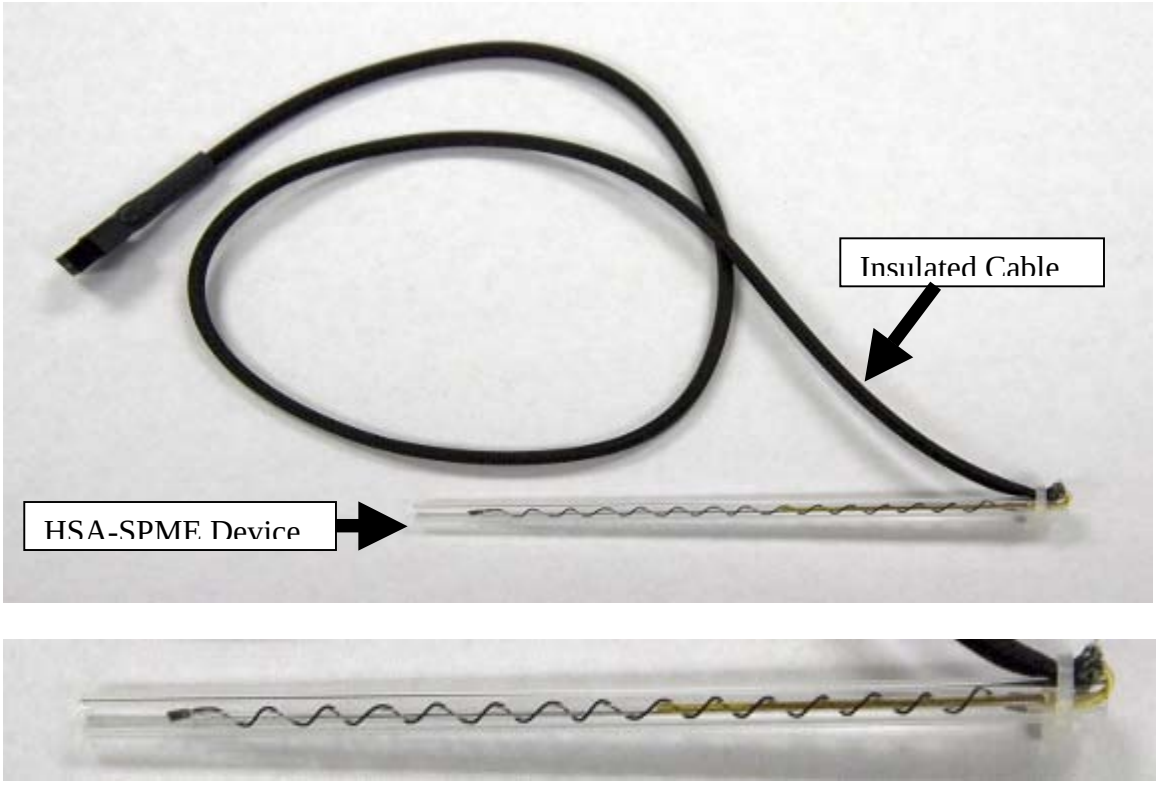

Figure 3.1. High surface area solid phase microextraction (HSA-SPME) device used in this study. The insulated cable contains the electrical leads necessary for resistive desorption and a type K thermocouple for temperature feedback.

The Carboxen-PDMS VOC collection phase has demonstrated good durability relative to PDMS-only coating when air sampling at relatively high flow rates [7-9]. Although Carboxen-PDMS can be subject to competitive sorption effects, these effects are minimized with the use of short sampling times, such as those used with the HSA-SPME method [7,8].

The HSA-SPME device used is shown in Figure 3.1. The specifics of the design were deliberate, as explained by Ramsey *et al.*, to take advantage of SPME mass uptake principles when the boundary layer surrounding the polymer is reduced [6-8]. The length of the wire used allows for ten times greater polymer surface area relative to a commercial SPME device (81 mm^2 vs. 8.1 mm^2) [6,7], while the outer borosilicate glass

tube (145 mm length x 4.8 mm o.d. x 3.0 mm i.d.) confines airflow past the wire into the zone directly adjacent to coated wire. It is this location in which the boundary layer exists, which is the zone adjacent/parallel to the fiber coating, and is the region where interaction between the sorbent layer and the sampled chemical compounds occurs [11]. As Bartelt and Zilkowski, as well as Ramsey *et al*. demonstrated, dynamic flow using faster flow rates over a SPME coating can increase mass uptake per unit time into the fiber by minimizing this layer [7,12]. The use of fast flow over the larger HSA-SPME surface area was found by Ramsey to achieve significantly higher mass uptake per unit time relative to SPME and previously used dynamic SPME methods.

3.2.4. Sampling Methods and Conditions

Three air sampling methods were used in this work. The first used non-exhaustive HSA-SPME sampling at 4 $L \cdot min^{-1}$ from a Tedlar® bag containing a known concentration of DIMP. The second method, tandem HSA-SPME, was the same as the first method except two HSA-SPME devices were used in series to provide double the surface area relative to the single HSA-SPME device method. The third method used exhaustive direct sampling (at a 0.585 $L \cdot min^{-1}$ flow rate) of DIMP from a Tedlar® bag with the Dynatherm automated chemical environment monitor (ACEM) Model 9305 pre-concentration module (CDS Analytical, Inc., Oxford, PA) and represents the control for the study. In the third method, active sampling loads a sample tube containing 200 mg of Tenax TA® sorbent material. Sampling for HSA-SPME was performed by drawing air at a set flow rate over the coated HSA-SPME element(s) using a small, battery-powered personal air sampling pump (GILAIR-5, Sensidyne/Gilian, Clearwater, FL) connected by

plastic tubing to the outlet of the device(s). Flow rate calibration was performed before and after collecting each sample using a primary standard calibrator (Bios International Corporation, Butler, NJ).

Additionally, a small amount of liquid material (2-DES) was sampled from the headspace above contaminated cloth at a flow rate of 4 $L \cdot min^{-1}$ to demonstrate the versatility of the HSA-SPME sampling device. The HSA-SPME device was connected with plastic tubing to a fitting present on the cap of the jar. Given the relatively small volume of the sampling jar containing the contaminated cloth, and the relatively high flow rates of the HSA-SPME method, pressure equalization from the surrounding atmosphere was required to permit sufficient air flow through the jar. Slightly unscrewing the cap of the jar permitted makeup airflow through the jar's headspace, allowing the operation of the HSA-SPME device at the 4 $L \cdot min^{-1}$ flow rate.

3.2.5 HSA-SPME Desorption

Automated, feedback controlled resistive heating of the HSA-SPME element was used for this study. A software adjustable heater control circuit board was used to desorb trapped analyte into the ACEM 9305 pre-concentration module (RVM Scientific, Santa Barbara, CA). This delivered a known, reproducible amount of power to the HSA-SPME element via the electrical leads connected to the nickel alloy wire of the device [13]. Desorption temperature for analyses was 250 ^{o}C. As shown in Figure 3.1, a type K thermocouple inside each HSA-SPME device and connected to the heater controller board provided temperature feedback.

Conditioning of the HSA-SPME element was performed initially by resistively heating the element to 300 ^{o}C for 30 minutes. High purity Helium (99.999%) (AirGas Specialty Gases, Radnor, PA) was passed through the device at 200 mL·min^{-1} during heating. Blanking of the HSA-SPME was performed between analyses, and at the beginning of each day of use, as carryover was present without the use of blanking between analyses. Desorption temperature when blanking or when performing analyses was set at 250 ^{o}C. The blanking procedure consisted of resistively-heating the HSA-SPME element in triplicate, then allowing it to cool to room temperature prior to sampling each time. This method was found to provide complete removal of analyte from the polymer coating, as determined by the absence of any detectable compound.

3.2.6 Pre-Concentration Module (ACEM) Operating Conditions

For all analyses, the standard temperatures used for air sampling into the ACEM were 40 ^{o}C for the sample inlet, 200 ^{o}C for the valve oven, and 40 ^{o}C for the sample tube. The system was loaded at the maximum flow rate where the sample pumping rate through the sample tube matched the flow rate set point (0.585 L·min^{-1}). Following the sample tube loading and a 1 minute purge flow, the ACEM valve system connected the flow path of the sample tube to the focusing trap, followed by thermal desorption of the sample tube at 250 ^{o}C for a period of 3 min. The temperature of the focusing trap was held at 40 ^{o}C, and subsequent desorption of the focusing trap occurred at a temperature of 250 ^{o}C for a period of 3 min, once the focusing trap flow path was connected by valve switching to the GC column.

3.2.7. Analytical Instrumentation

The ACEM pre-concentrator module was coupled to an LTM-GC/MS system (RVM Scientific, Santa Barbara, CA), retrofitted to a standard Agilent 6890N GC oven through an insulated 3-meter statically heated (225 ^{o}C) transfer line. A DB5-MS capillary column (30 m length x 0.25 mm ID x 0.25 μm film thickness) (J & W Scientific, Folsom, CA) was used for all analyses. The GC temperature programming was initiated at 40 ^{o}C (30 s hold time) with subsequent temperature ramping of 5 ^{o}C·min^{-1} to a final temperature of 130 ^{o}C. High purity Helium was used as carrier gas, with a constant flow rate of 1.0 mL·min^{-1}.

A commercially available 5973 quadrupole MS detector (Agilent Technologies, Wilmington, DE) was used for analyses. Electron ionization (70 eV) was used and mass spectra were collected in scan mode over the range of *m/z* 95 - 180 when analyzing DIMP, and *m/z* 70 - 180 when analyzing 2-DES. Extracted ion chromatograms (*m/z* 97, 123 and 165 for DIMP and *m/z* 72, 89 and 114 for 2-DES) were examined following analysis to allow for improved detection of these analytes. Sample retention characteristics and mass spectra were stored and analyzed using Agilent Chemstation software (Version D.00.00.38).

3.2.8. Calibration Procedure

To gauge mass uptake onto the sorbent material, and the sensitivity of the sampling methods used in this study, liquid calibration curves were created for both compounds by direct injection of a known mass (ng) into the sampling port of the ACEM 9305. The GC peak areas produced from the sampling and analysis methods used were

compared with the resulting calibration curves to provide an indication of mass uptake for the HSA-SPME method.

3.2.9. Statistical Analysis

A two-way analysis of variance (ANOVA) was used in the determination of comparative significance between the two different sampling methods when using the four different fixed sampling times, as well as when using the two different methods with the three fixed sampling volumes. A three-way ANOVA was used to ascertain significance in capture efficiency for the three different methods used in tandem HSA-SPME analysis. If a significant interaction was noted among the factors, then pair-wise comparisons by Tukey's adjustment were used. The statistical analyses were performed using the Statistical Analysis Software (SAS) (SAS Institute, Inc., Cary, NC) program. In cases of unequal variance among means, average abundance levels were adjusted to natural log scale (with subsequent antilog of the results) to better conform to the assumptions of equal variance when using ANOVA.

3.3 Results and Discussion

3.3.1 DIMP Air Sampling with HSA-SPME

Figure 3.2 shows the redundant results of sampling a Tedlar® bag containing a nominal 13 ppb_v concentration of DIMP while using either the single HSA-SPME device method or the ACEM only methods for fixed sample volumes. As expected, the higher flow rate used for HSA-SPME and the lower overall efficiency of this sampler resulted in significantly less total mass uptake compared to the lower flow rate used for the ACEM

only method. The area counts for DIMP when using the HSA-SPME method were found to be approximately 20% of the ACEM only method results for a given sample volume.

The relative inefficiency could be attributed to not only the higher flow rate used, but also to the potential losses incurred in the two step process that accompanies each HSA-SPME analysis. The first step involves uptake of analyte onto the HSA-SPME device and is affected by the air flow and environmental conditions during collection, and the affinity of a particular chemical for the specific SPME coating. Inefficiency could result if a compound either does not interact with the HSA-SPME coating, or a target molecule may interact with the coating but then diffuse back into the surrounding airstream.

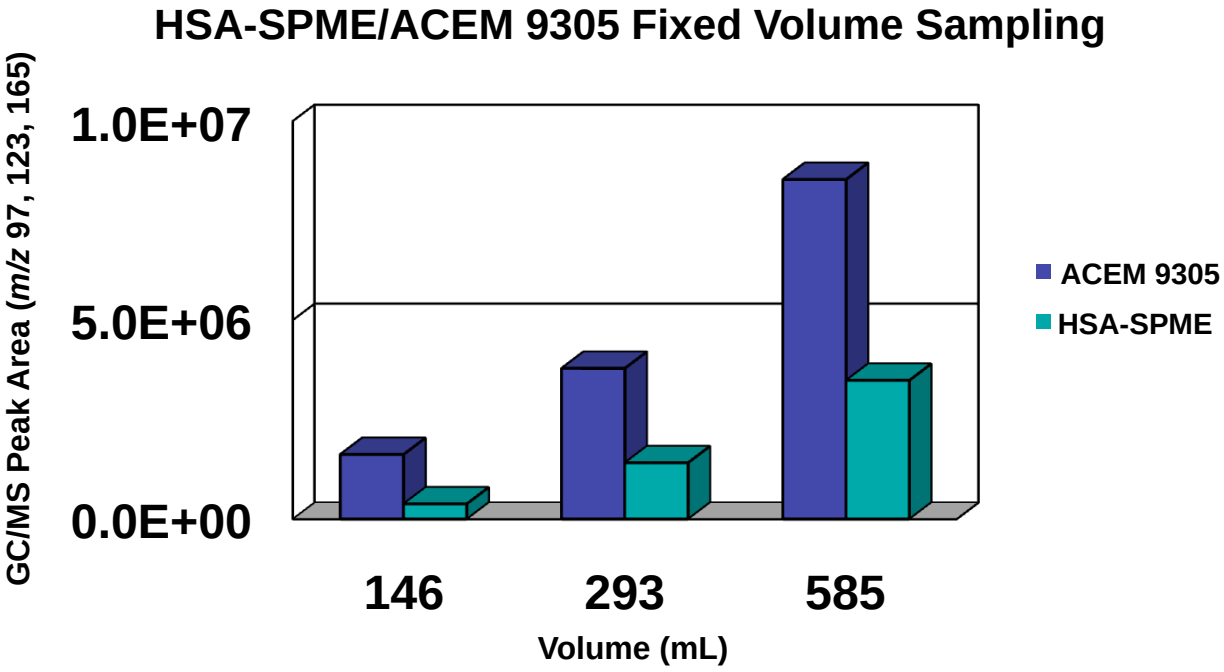

Figure 3.2. Results from fixed volume comparison of the HSA-SPME (single device) and the ACEM methods (13 ppb_v DIMP). As expected, the ACEM displays greater capability than HSA-SPME at fixed volumes, as it represents exhaustive sampling ($p < 0.0001$). Relative standard deviation for the ACEM method ranged from 1.92 to 7.32 % and from 1.86 to 10.46 % for the HSA-SPME method. GC/MS peak areas for triplicate averages of the sum of *m/z* 97, 123 and 165 ion current.

The second step, in which analyte is desorbed onto the ACEM sample tube after resistively heating and flushing the coating of the HSA-SPME device with helium flow, may have resulted in the loss of analyte due to incomplete desorption or other unexplained sample loss.

While the overall extraction inefficiencies of the HSA-SPME method resulted in a greater comparable mass uptake per fixed volume for the ACEM only method, the HSA-SPME method had a distinct advantage in speed of collection, due to the greater mass uptake rate per unit time for this approach. Flowing 4 L of sample volume through the sample tube using the ACEM only method required 6.8 min, compared to 1 min for the same sample volume using the HSA-SPME method.

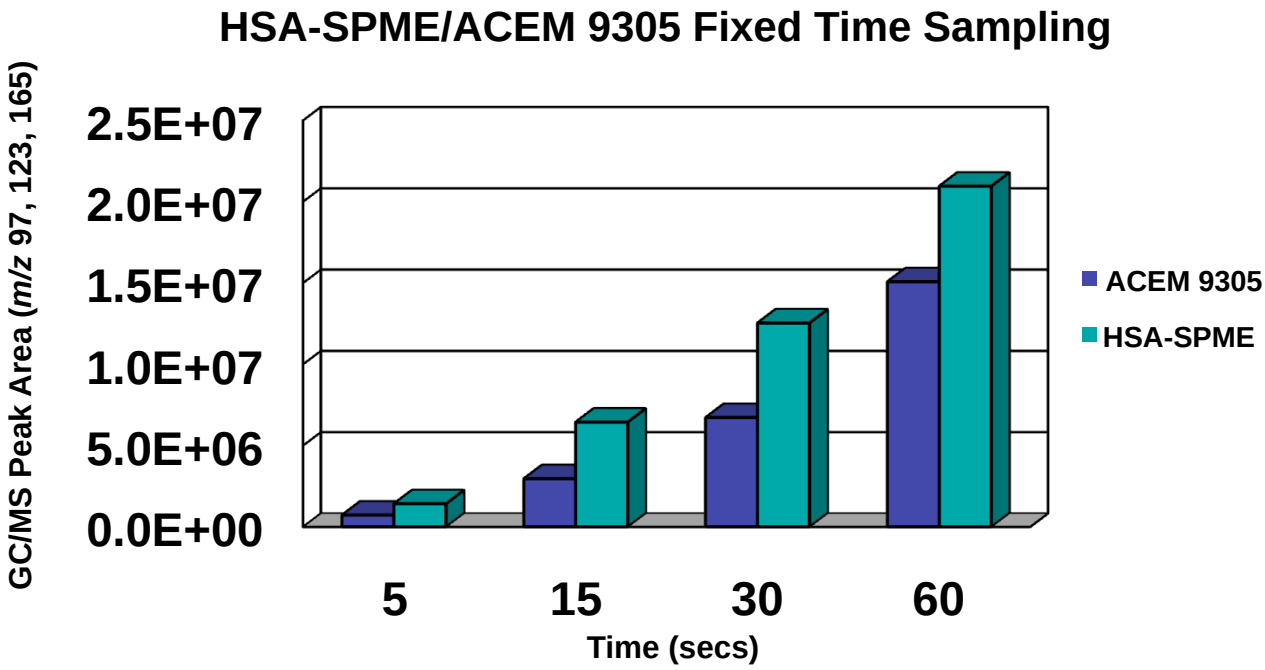

Figure 3.3. Results for HSA-SPME (single device) and ACEM methods at discrete sampling times (13 ppb_v DIMP). Extraction for HSA-SPME is observed to produce significantly higher mass uptake per unit time (p value < 0.0001) compared to the ACEM method which had lower flow rates. Relative standard deviation for the ACEM method ranged from 1.92 to 6.73 % and from 1.80 to 6.90% for the HSA-SPME method. GC/MS peak areas for triplicate averages of the sum of *m/z* 97, 123 and 165 ion current.

Although the extraction efficiency for the HSA-SPME method is low when sampling to achieve a discrete volume, the extraction efficiency per unit time for this sampling approach leads to greater GC/MS peak area counts when the total volume of air sampled is not considered. As shown in Figure 3.3, the greater analytical response corresponds with use of a higher flow rate for the HSA-SPME method relative to the ACEM only method, despite the lower trapping efficiency on a volumetric sampling basis. Volumes of 333 mL, 1000 mL, 2000 mL and 4000 mL of sample using HSA-SPME were delivered over the surface of the polymer device in 5, 15, 30 and 60 seconds, respectively. In contrast, the ACEM only method loaded sample mass from nearly 7 times less air sample volume for the same fixed time periods when at maximum optimum flow. The ACEM only method with a Tenax TA® 20:35 mesh size sample tube containing 200 mg of sorbent did not allow for flow rates higher than 0.6 $L \cdot min^{-1}$ due to pressure drop across the sample tube.

Results using fixed sample times for the two methods, as observed in Figure 3.3, yielded a 50% greater chromatographic response for the same concentration of DIMP when using HSA-SPME relative to the ACEM only method at 5, 15, and 30 s, with an approximately 35% greater response at 60 s. The reduced comparative response for the HSA-SPME method at the highest sample time period (60 s) suggests that the rate of mass uptake for the HSA-SPME method rapidly reaches a maximum during the first 30 s of sampling at the 4 $L \cdot min^{-1}$ flow rate and then levels off between 30 and 60 s sampling time at this flow rate. Results suggest that given the significantly higher surface area of the sorbent material used in the exhaustive ACEM method relative to the coating of the non-exhaustive HSA-SPME device, sample times of several minutes could conceivably

Concentration ppb_v	Sampling Duration (s)	Average Area Count	% RSD
13	5	1,929,344	8.26
3.5	5	403,518	2.93
1.0	10	193,666	5.37
0.75	15	92,226	12.45

Table 3.1. Tandem HSA-SPME results for DIMP at different concentrations (ppb by volume) and sampling durations. DIMP was detectable at all four concentrations. Liquid injections of DIMP into sample tube of ACEM were performed at 32.7 ng, 7.3 ng, 5.6 ng and 4.9 ng, respectively. GC/MS peak areas for triplicate averages of the sum of *m/z* 97, 123 and 165 ion current.

result in greater analytical response with the ACEM only method relative to the HSA-SPME method, as the HSA-SPME coating material surface sites would eventually become completely filled.

Efficiency results for tandem HSA-SPME sampling at 13 ppb_v, 3.5 ppb_v, 1 ppb_v, and 0.75 ppb_v at 5, 5, 10, and 15 s sampling times, respectively, provided a better than 3:1 signal to noise response for DIMP in all cases. Table 3.1 displays the chromatographic response (in average area counts) for each concentration when using the tandem HSA-SPME technique compared to direct liquid injection of DIMP into the sampling port of the ACEM. The ACEM only method was unable to detect DIMP at 3.5 ppb_v or lower when sampling for the only 5 s. Results of the tandem HSA-SPME method in comparison with direct liquid injections (assumed as 100% efficient) were below 10% for all 4 concentrations in terms of average area count. Describing the efficiency of the HSA-SPME method, Ramsey *et al*. used a term from a mathematical model developed by Bartelt and Zilkowski regarding non-equilibrium sampling of volatiles with a straight traditional 1 cm long SPME device [2,8]. In their research, Bartelt and Zilkowski found

that when using an absorptive dynamic SPME sampling method with active parallel flow, the initial loading rate of the fiber may be described by Equation 3.1:

(3.1) $$(dM_{fiber}/dt)_{entry} = EFC_{air}$$

The E value is a unitless efficiency factor ranging from 0 to 1, F is the volumetric flowrate over the SPME coating in $mL \cdot min^{-1}$, and C_{air} is the steady state concentration of analyte in the sampled air .

Bartelt and Zilkowski found that for short duration sampling periods at constant concentration, the loading rate may be greater with faster sample flow rates, even though faster flow rates result in a lower E term [11]. In this work, the E value was determined to be 0.015. When using this E value and sampling a 13 ppb_v (0.098 $ng \cdot mL^{-1}$) concentration for 5 s using the tandem HSA-SPME method, the average area count which corresponded with the mass result was comparable to liquid calibration curve area count results for 0.5 ng, while the mass of DIMP in the air volume sampled was 33 ng.

To further examine the mass uptake for the tandem HSA-SPME method, mass uptake of each respective device was measured. Instead of desorbing the contents of both devices onto the ACEM sample tube prior to a single sample tube desorption and subsequent analysis as was normally done with the tandem method, each respective device was desorbed and analyzed separately and the analytical response of the first device was compared to the analytical result of the second device.

	First Device Only	Second Device Only	Sum of Separate Analyses	Single Tandem Analysis
Mean Area Count	867,515	458,220	1,325,735	1,375,600
% RSD	7.67	7.72	2.35	2.57

Table 3.2. Mass uptake for the front (first device) and back (second device) of tandem HSA-SPME sampling for 15 ppb_v DIMP; separate analyses. The sum of independent results for the front and back devices was then compared to the results of analyzing both devices in a single run (single tandem analysis). GC/MS peak areas for triplicate averages of the sum of *m/z* 97, 123 and 165 ion current.

When performing these analyses with two different concentrations, 15 ppb_v and 5 ppb_v, it was observed that the chromatographic response (in area counts) of the second HSA-SPME device in the tandem was 45% to 55% of the chromatographic response of the first HSA-SPME device. The difference in area counts from the two devices was found to be significant (p value < 0.01). When these values were added, the total was not significantly different from the total produced when the two devices were loaded and analyzed together as a single run (p value > 0.2721). These results suggest that the first device in series is depleting the sample mass in the volume flow prior to reaching the second device in series. Results are displayed in Table 3.2.

3.3.2 Headspace Sampling of Contaminated Clothing

The VX degradation compound, 2-DES, was used to simulate an incident involving dispersal of liquid chemical warfare agent into the air and onto the clothing of bystanders in the immediate area. Figure 3.4 illustrates the results from the tandem HSA-SPME method compared with direct liquid injection of 2 uL of a 25 nanogram per uL concentration onto the sample tube of the ACEM. This aspect of the study was employed

to determine the capability of the HSA-SPME in detecting trace contamination of a liquid chemical warfare degradation product less volatile than Sarin or Soman.

When using the tandem HSA-SPME method to sample 2-DES at a temperature simulating a hot summer day (30 ^{o}C), an analytical response of 10^6 area count was produced after a 15 s vapor headspace sampling event (Figure 3.4). This result is of significance due to the likelihood of clothing being contaminated in such an event, as well as the general inability of commercially available and widely used field portable chemical detection systems such as the Hapsite® to detect trace levels of semi-volatile compounds with retention indices of greater than 1200-1300 in the headspace above a respective liquid contaminant. Many CWAs and their degradation compounds are semi-volatile, with retention indices of greater than 1200, and cannot be detected by the Hapsite®, despite several minutes of on-site sampling in stand-alone mode.

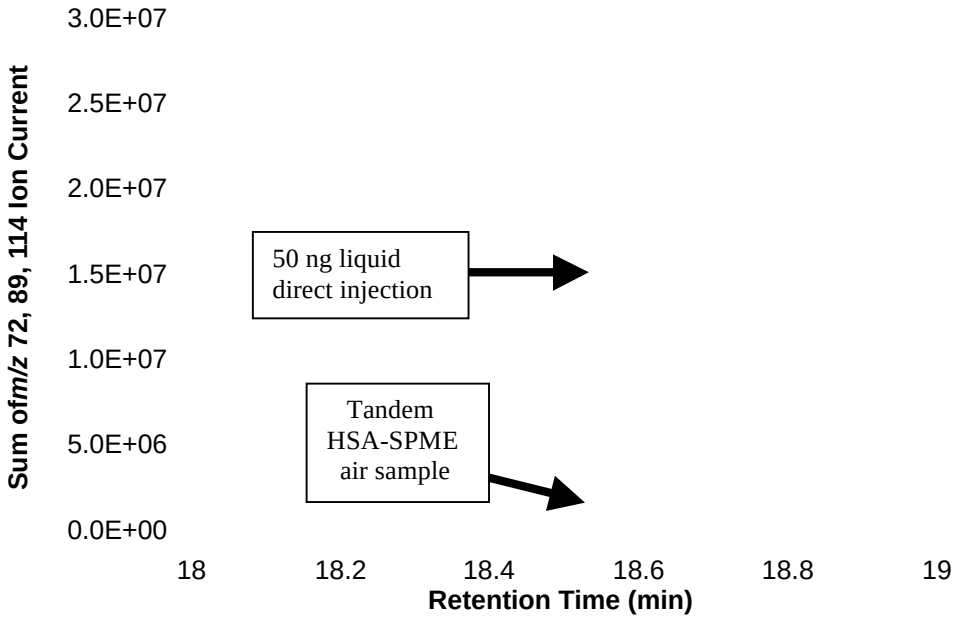

Figure 3.4. GC/MS extracted ion chromatogram (sum of *m/z* 72, 89, 114) of a 50 ng liquid direct injection into the ACEM sample tube and a 15 s HSA-SPME headspace sample at 4 L·min^{-1} of a cotton cloth contaminated with 50 ng of the VX degradation compound 2-DES.

3.4 Conclusion

Results of this study indicate the potential utility of the rapid and highly-portable HSA-SPME collection technique for trapping trace VOCs, as well as allowing for a high level of method sensitivity when coupled with GC/MS instrumentation. The HSA-SPME method demonstrated its versatility as an air sampling approach as well as a fast, effective means of providing headspace sampling of trace quantity liquid-contaminated articles. Significantly greater mass uptake per unit time was observed with the HSA-SPME method when compared to a laboratory accepted pre-concentration method, as determined by analytical responses of 35% to 50% greater analyte abundance levels when sampling a low ppb_v concentration of DIMP. When the tandem HSA-SPME method was employed in the active sampling of a sub-ppb_v concentration, detection was accomplished for DIMP using the MS full scan mode after only a 15 s sampling time. Also, this collection method can potentially provide emergency responders with the fast and definitive results required to protect human health when dealing with liquid contamination with highly toxic CWAs.

As a forensic tool, use of the HSA-SPME method may provide the forensic scientist with a potential analytical means of corroborating canine scent detection. Given improved mass uptake per unit time compared with traditional methods which are not used at flow rates in the liters per minute range, as well as the wide affinity range for polar and non-polar chemicals of coatings like Carboxen-PDMS, the HSA-SPME method may prove helpful in populating human scent volatiles databases. Databases such as these are currently being developed by the Federal Bureau of Investigation to track missing persons or to determine the location of human remains. The ability to improve

sensitivity levels for the various volatiles through the use of high sampling flow rates may also allow for an approach to corroborate the canine model in terms of speed and sensitivity. Field sampling of trace VOCs in a rapid and accurate fashion can provide increased confidence and support for courts of law.

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Chapter 4

Influence of Fast Temperature Program Rates on Chemical Warfare Agent Gas Chromatography Retention Index Values

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Abstract

This work examines the impact of fast temperature programming gas chromatography relative to a rate of 10 °C·min^{-1} when analyzing 5 chemical warfare agents and one chemical warfare agent degradation product. Pronounced shifts in retention index (RI) values with respect to *n*-hydrocarbon reference standards were observed for several of the compounds analyzed using temperature ramp rates as high as 120 °C·min^{-1}. This was explained by considering the increase in time-weighted average column temperature between injection and elution at higher temperature programming rates, and comparison to retention factor (*k*) values obtained from isothermal analyses of the same compounds. The impact of fast temperature programming rates on RI values was reproducible, and linear temperature program retention characteristics (k'_{LPTGC}) versus time-weighted average column temperature from time of initial column migration to time of elution were closely related to *k* versus isothermal analysis temperature.

4.1 Introduction

Field portable chemical detection equipment can play an important defensive role in protecting public health, civilian emergency responders, and military forces. To meet this defensive need, capabilities for rapid detection and identification of harmful chemicals must include identification of trace level contaminants present in a variety of complex environmental matrices. Gas chromatography with mass spectrometric detection (GC/MS) is a well-established and sensitive method that can accomplish this in many cases by providing a chemical compound's retention time and corresponding mass spectrum [1,2]

In GC/MS analysis, identification of an unknown is frequently completed by mass spectrum matching against reference spectra found in a digital library, such as that developed by the U.S. National Institute of Standards and Technology (NIST) [3]. An apparent match to a library mass spectrum can be of great value in identifying unknown chemicals, but is often complicated by a number of factors. The use of retention time (t_R) adds an important dimension in identification by GC/MS, but absolute retention time values will differ from one instrument to another, even when chromatographic conditions are standardized as closely as possible [1]. If GC/MS is to be fielded in the defensive roles mentioned, the use of both reproducible retention data comparisons and mass spectrum match information provides improved accuracy in identifying the chemicals present. In situations where significant risks could exist with misidentification, this accuracy is especially important. Database information derived from retention characteristics can be useful when standardized methods are available that allow comparisons between data collected from different instruments [4,5]. When analysis is

completed using non-standard methods, the impact of altered GC conditions must be known.

The use of retention indices (RIs) permits comparison of t_R data obtained from one instrument to those from another when the same stationary phase material and important analysis conditions are used in both cases. Van den Dool and Kratz [6] proposed a method for calculating RI values (Equation 4.1) for analyte mixtures having a wide range of volatilities:

(4.1) $$RI = 100\left(\frac{t_{R(z+n)} - t_{R(z)}}{t_{R(z+n)} - t_{R(z)}}\right) + 100z$$

Here, t_R is the uncorrected retention time for the analyte of interest, n is the difference in carbon number between the reference materials (frequently members of a homologous *n*-hydrocarbon series) eluting before and after an unknown analyte, $t_{R(z)}$ is the retention time of the reference compound eluting before the unknown analyte, and $t_{R(z+n)}$ is the retention time of the reference compound eluting after the unknown analyte. When straight chain aliphatic hydrocarbons are used as reference standards, z is the number of carbons in the early eluting reference peak. This approach allows the use of linear program temperature GC (LPTGC) with a mixture of reference compounds having widely different volatilities to provide RI data from a single analysis, instead of previous methods that would require several isothermal runs.

Considerable amounts of LPTGC RI data based on typical laboratory GC operating parameters are available for high-concern chemicals such as chemical warfare agents (CWAs) and related materials [7,8]. The standard temperature programs for the

analyses tabulated by Kostiainen [8] include a brief isothermal period at 40, 50, or 60 ºC, followed in each case by ramping at 10 °C·min^{-1}, with typical analysis time of around 20 min. However, fast analysis times are desirable for field detection of high concern analytes. Several approaches that facilitate faster GC separations include rapid column heating and the use of high velocity H_2 carrier gas. Smith *et al.* demonstrated separation of CWA mixture components ranging in molecular weight from *m/*z 140 (sarin) to 466 (T2 toxin) with a field-portable GC/MS system using both of these approaches. A low thermal mass (LTM) GC column assembly was resistively heated to produce temperature ramping rates of 200 °C·min^{-1}. This was used in conjunction with H_2 carrier gas at a linear velocity of 100 cm·s^{-1} to provide analysis times of less than 5 min [9].

The use of resistive GC column heating methods based on the approach of Sloan *et al.* [10], is now routinely possible. Small LTM GC components are commercially available at reasonable cost and can be retrofitted to an existing air bath GC oven to provide fast column heating and cooling. The small LTM GC assembly may be mounted on the traditional GC convection oven door with transfer lines between the externally-mounted LTM GC column and both the injector and the detector passing through the convection oven for static heating.

The retrofit approach retains the convection oven of the GC system as a transfer line heater, but additional reductions in overall system size and power consumption are possible with the removal of the convection oven. This has been done for at least two commercially available GC/MS systems designed with a resistively heated LTM GC module for use in the field. Further trends towards this GC column heating method are likely for new field-portable instruments since it allows for rapid LPTGC ramping with

low power consumption and small overall size. With these trends in mind, the effect of fast GC programming on RI values for target analytes should be addressed. The effects of increased LPTGC ramp rates on RI values, and the basis for any observed changes in RI values must be understood if fast LPTGC ramping rates are to be fully exploited for rapid analyses of high concern analytes.

In this work, GC/MS analyses of several CWAs and a high molecular weight CWA degradation product were completed, and changes to RI values from using LPTGC ramp rates between 5 and 120 °C·min^{-1} are described. To examine the link between temperature and elution changes, retention factor (*k*) values were calculated following varied isothermal analysis conditions, and the analogous k'_{LPTGC} values were calculated for the same analytes using the range of LPTGC ramp rates as noted above.

4.2 Materials and Methods

4.2.1. Instrumentation

Analyses were performed using both a standard Agilent 7890A gas chromatograph with a 5975C quadrupole MS detector (Agilent Technologies, Wilmington, DE), and a field portable GC/MS system in which the typical convection air bath oven of an Agilent 6890/5973 combination was replaced with a resistively heated column (RVM Scientific, Santa Barbara, CA) as originally described by Smith *et al.* [9]. Ultra high purity (5.0 UHP) H_2 was used as the carrier gas for the field-portable instrument at 100 cm·s^{-1} linear velocity (constant pressure, initial velocity), while 5.0 UHP He was used as the carrier gas for the standard laboratory GC/MS system, at a linear velocity of 44 cm·s^{-1} (constant velocity mode) for isothermal analyses. The

columns used in these instruments had identical dimensions (30 m, 0.25 mm I.D., and 0.25 μm d_f) and similar stationary phase material. The laboratory GC/MS system operated with HP-5MS stationary phase (Agilent Technologies) while the GC column of the LTM GC/MS system was DB-5MS (J & W Scientific, Folsom, CA). The injector temperature for both instruments, as well as the injector transfer lines for the instrument equipped with the resistively heated GC column were set at 250 ^{o}C, while the MS transfer line temperature of both instruments was set at 250 ^{o}C. In both cases, standard electron ionization (70 eV) was used, and mass spectra were collected over the range of *m/z* 35 - 300 and *m/z* 45 - 300 respectively for the lab-based and LTM GC/MS systems. Sample retention characteristics and mass spectra were stored and analyzed using Agilent Chemstation software.

4.2.2 Chemicals

Consecutive C_8-C_{21} *n*-alkanes were used as reference standards for determination of RI values for the CWA-related compounds studied. The analytes studied included isopropyl methylphosphonofluoridate (GB, or sarin), pinacolyl methylphosphonofluoridate (GD, or soman), bis (2-chloroethyl) sulfide (HD, or sulfur mustard), cyclohexyl methylphosphonofluoridate (GF), O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothiolate (VX), and bis (diisopropylaminoethyl) disulfide ($(DES)_2$). All CWA analytes were handled only by licensed personnel under controlled conditions at Defence R&D Canada – Suffield (DRDC Suffield) Ralston, AB.

The *n*-alkane reference hydrocarbons were purchased commercially (Aldrich, Milwaukee, WI, USA), and the CWAs were synthesized by DRDC Suffield

personnel, or were available to them from existing material. Prior to use, a stock solution of each CWA compound was prepared in methylene chloride from the standard CWA materials available at the Canadian National Single Small Scale Facility. The purities of the CWA analytes were verified by GC–MS to be 96%, 98%, 92%, 91%, and 75% respectively for GB, GD, GF, HD, and VX.

The degradation product studied in this research, $(DES)_2$, was synthesized using methods and materials previously reported [11]. This compound is known to be a common indicator for VX contamination [11,12], resulting from breakage of the sulfur-phosphorous bond of the parent VX molecule, and subsequent oxidation of the resulting thiol which readily occurs in the presence of O_2. The identity of the synthetic standard for this compound was confirmed by GC/MS analysis with methane and ammonia chemical ionization, ^{1}H and ^{13}C NMR, and by comparison of the compound's EI-MS spectrum and RTI value to data available in the literature using the same GC parameters as in the cited reference [13].

4.2.3 Samples and Sample Introduction

All analyses where LPTGC ramp rate was varied for RI calculations were completed using the GC/MS instrument designed for field use, while all isothermal analyses and analyses where carrier gas type and linear velocity were varied were completed using the laboratory-based convection oven GC/MS system. All analyses were completed with liquid injection of CWA-related and reference hydrocarbon analytes dissolved in methylene chloride. Triplicate sampling was conducted for

each data point and all reported results represent the average of the triplicate samples.

4.2.4 GC Temperature Conditions

The Organization for the Prohibition of Chemical Weapons (OPCW), established to ensure treaty compliance with the Chemical Weapons Convention (CWC), conducts sampling and analysis for verification of chemical weapons disarmament. The OPCW uses the following recommended temperature program for verification: initial temperature of 40 ^{o}C for 1 min, followed by a heating rate of 10 °C·min^{-1} to 280 ^{o}C for 10 min [8]. The GC temperature programs used in this study were predicated on fast GC-MS analyses of CWAs, and a range of GC column heating rates were thus investigated. Initial GC column temperature was held at 40 °C for 30 s, and temperature ramping rates following this initial period ranged from 5 to 120 °C·min^{-1} with final temperature of 300 °C. Temperatures for isothermal analyses completed using the laboratory GC/MS system included a range empirically determined to provide definable peaks that could be integrated with the Agilent Chemstation software used for data analysis.

For LPTGC analyses with the resistively heated column, potential temperature lag was examined by calculating the assumed straight-line temperature parameters of the LPTGC profile and then checking for lag with a diagnostics board connected to the heater controller board of the field-portable GC system. A data-logging multi-meter recorded the second-by-second voltage readings related to resistance in the temperature sensing wire of the LTM GC module during an LPTGC run using proprietary software (Metex Corporation, Seoul, S. Korea). The column's actual temperature within the LTM module was obtained with this information using a conversion equation from the developer of the

LTM GC module. No appreciable temperature lag was noted, as the difference between the temperature set points and actual temperatures over the 40 ^{o}C to 300 ^{o}C range was less than 4%.

4.2.5 Isothermal and LPTGC Retention

Under simple isothermal conditions, retention for a given analyte in gas-liquid chromatography is fundamentally based upon the distribution constant:

(4.2) $\square = C_S C_G$

where C_S and C_G are respectively the analyte concentrations in the liquid film and in the carrier gas under the specified conditions. As shown in Equation 4.3 the retention time of an analyte (t_R) relative to the retention time of an unretained analyte (t_M) is described by the retention or capacity factor k. In this work, the term k'_{LPTGC} refers to the value obtained using the same measurements of t_R relative to t_M and Equation 4.3, but with LPTGC conditions.

(4.3) $\square R - \square M \square M = \square$

The relationship between k and K is defined by accounting for the phase ratio β, which is a measure of the relative volumes of the mobile and stationary phases.

(4.4) $K = k\beta$

Where two discrete temperatures could be used for a single analysis, assuming instantaneous change from one temperature to the second, the retention characteristics would depend on the time (t_n) spent at each *K* condition.

(4.5) $$\beta R - M M = 1 1 + 2 2 1 + 2$$

Approximating continuously changing temperature conditions found in LPTGC, if n discrete temperatures are used in succession, then similarly,

(4.6) $$\beta R - M M = 1 1 + 2 2 + \ldots n n 1 + 2 + \ldots n$$

While several mathematical approaches have been developed previously to relate isothermal and linear temperature program retention values [14-19], our goal was to demonstrate that RI changes could be explained by a simple metric. The value of *K* for a given analyte in a specified stationary phase may be altered solely by temperature change, although the relationship is not linear. For isothermal analysis, the column temperature (T_C) used is inversely proportional to log(*k*) as shown in Equation 4.7 [20].

(4.7) $$\log \propto 1Tc$$

In the case of LPTGC analysis, the proposed use of Equation 4.7 requires that the value of T_C must be a time-weighted average column temperature to which an analyte is subjected between injection and elution. This can easily be calculated by integrating the

corresponding area under the GC column temperature curve, and dividing by the elapsed time to elution. Values of k'_{LPTGC} at the various time-weighted average column temperatures can then be compared with those for k obtained under isothermal GC conditions. Although K is not directly measured, continuous changes to K during LPTGC analysis would be the underlying cause of retention changes.

The effect of a higher initial temperature on k'_{LPTGC} values for analyses with varied LPTGC temperature ramping rates was also examined. At cooler initial LPTGC temperatures, higher molecular weight compounds will tend to partition much less to the gas phase, resulting in a higher observed k'_{LPTGC} value due to relatively large values of t_R in Equation 4.3. For very large analytes it is possible for no movement to occur initially until the temperature is increased sufficiently during LPTGC to cause analytes to partition to the gas phase. The ability to explain RI changes based upon time-weighted average column temperatures for larger analytes with less initial LPTGC volatility was examined using the relatively high molecular weight compound $(DES)_2$. In addition to the typical LPTGC analysis start temperature of 40 °C, values of k'_{LPTGC} and corresponding time-weighted average column temperatures for this compound were also obtained from linear temperature programs starting at 150 ^{o}C, an initial temperature high enough for $(DES)_2$ to have some gas phase activity.

Previous work by Podmaniczky *et al.* explored various aspects of isothermal and LPTGC conditions as they relate to fundamental thermodynamic data [16], isothermal RI values [17], LPTGC RI values [18] and k'_{LPTGC} [19]. Observed values of k'_{LPTGC} for $(DES)_2$ in the current work were compared to predicted values using the calculation methods of these authors which are based upon isothermal k values [19].

4.2.6 Carrier Gas Velocity

Due to increased carrier gas viscosity as the column temperature is increased, adjustment was made for the lack of automated pressure control in the field-portable GC system. This was done by injecting air into the field-portable GC at isothermal temperature set points over a range of temperatures (40 to 300 ^{o}C, in 10 ^{o}C intervals). The resulting t_M values were used to provide a best-fit equation. The corresponding t_M' values thus obtained by using the time-weighted average column temperature with the best fit equation were used in calculations to determine k'_{LPTGC} values.

While the instantaneous value of K is only affected by temperature, carrier gas linear velocity affects all of the terms in Equation 4.3. Very fast linear velocities will cause LPTGC elution of an analyte more rapidly (thus at cooler column temperatures) due to lower values for both t_R and t_M. To demonstrate the effect of carrier gas linear velocity on the time-weighted average column temperature for specific analytes, analyses were completed with the laboratory GC/MS system using both H_2 and He carrier gas for LPTGC with a column temperature ramp rate of 10 °C·min^{-1}. For these analyses the starting column temperature was 40 °C, which was held for 30 s. Temperature ramping occurred until 250 °C and the column was held at the terminal temperature for 2 min to clean the column. Constant flow mode was used, with linear velocities of 40, 60, 80, 100, and 120 cm·s^{-1}. Average carrier gas linear velocity was verified by measuring t_M for air, with the constant flow rate adjusted as needed to arrive at the specified nominal velocities.

It was not possible to use H_2 with a velocity of 40 cm·s^{-1} as the high vacuum of the mass spectrometric detector causes velocities greater than this value even with the GC

column head pressure near zero. Thus to compare RI values with those in the literature collected using routine conditions [7,8], analyses with linear velocity of 40 $cm \cdot s^{-1}$ were completed using He carrier gas. Also, it was not possible to obtain data for He carrier with a velocity of 120 $cm \cdot s^{-1}$ as the highest programmed injector head pressure possible produces velocities less than this.

4.3 Results and Discussion

As shown in Table 4.1 and Figure 4.1, when temperature ramp rates greater than 10 $^{o}C \cdot min^{-1}$ were used, RI value changes were observed. Large shifts in RI values were not observed for GB and GD (GD not shown in Figure 4.1) when temperature ramping rates of between 5 and 120 $^{o}C \cdot min^{-1}$ were used, varying by only a few RI units on average. When both carrier gas linear velocity and LPTGC ramp rate are matched closely to conditions used previously to provide RI values [7,8] RI values are very close to those found in the literature (Table 4.2).

Shifts in RI values relative to standard LPTGC heating conditions were more pronounced for HD, GF, VX and $(DES)_2$. These shifts ranged from about 15-20 RI units for HD and GF to about 30-40 RI units or greater for VX and $(DES)_2$, respectively, when operating at the highest ramp rates. The direction of RI value deviation observed in this study agrees with the work of Kokko *et al.*, which showed an increase in RI values as LPTGC ramp rates were increased from 2 to 10 $^{o}C \cdot min^{-1}$ for VX [5]. In addition to the RI values calculated for each compound over the range of LPTGC temperature program rates used, Tables 4.1 and 4.2 provide comparisons to literature values for the analytes studied using standard 10 $^{o}C \cdot min^{-1}$temperature ramping rates [8,13,21,22].

The use of faster temperature program rates results in higher time-weighted average column temperatures relative to slower rates. Table 4.3 displays the calculated time-weighted average column temperatures for the most and least volatile compounds studied respectively, GB and $(DES)_2$, at the various LPTGC ramp rates used. When the analytes studied were subjected to higher time-weighted average temperatures produced by the faster ramp rates, retention characteristics showed similar trends as with higher isothermal column temperatures.

Table 4.1. Observed RI values for the analytes studied with high velocity H_2 carrier gas (100 $cm \cdot s^{-1}$ initial velocity) and LPTGC ramp rates from 5 to 120 $°C \cdot min^{-1}$ and published reference values collected using He carrier gas at LPTGC ramp rate of 10 $°C \cdot min^{-1}$.

Compound	**Reference**	5° C min^{-1}	10° C min^{-1}	20° C min^{-1}	40° C min^{-1}	60° C min^{-1}	80° C min^{-1}	100° C min^{-1}	120° C min^{-1}
GB	**823.8**[1]	814.0	814.9	815.4	815.8	816.0	816.9	817.0	816.9
GD[A]	**1045.2**[1]	1032.1	1032.7	1033.8	1034.9	1035.8	1036.4	1037.1	1037.8
HD	**1172.7**[1,2]	1169.2	1175.8	1177.9	1182.8	1186.1	1188.7	1190.2	1191.5
GF	**1208**[3]	1194.4	1196.7	1200.0	1203.5	1206.1	1208.4	1209.4	1210.8
VX	**1710.1**[1] **1705.0**[4]	1682.0	1688.4	1695.3	1702.7	1708.5	1712.5	1715.1	1718.3
$(DES)_2$	**2057.9**[4]	2049.0	2057.6	2068.2	2080.8	2088.4	2093.8	2097.5	2101.1

[A]Based on retention time for 1st eluting peak of the GD enantiomeric pair; [1]D'Agostino *et al.* [21], [2]D'Agostino *et al.* [22], [3]Organization for the Prohibition of Chemical Weapons (OPCW) database [8], [4]D'Agostino *et al.* [13]. Coefficient of variation values calculated from replicate samples were <1% in all cases. Standard deviation did not exceed 1.49 for all compounds over the range of programmed heating rates.

The increase in time-weighted average column temperature due to higher LPTGC ramp rates caused values of k'_{LPTGC} to decrease with each successive increase in temperature ramp rate, similar to the inverse relationship between isothermal temperature and k shown in Equation 4.7 [20, 23]. Elution order changes were observed as both isothermal analysis temperatures and LPTGC ramp rates were varied. Elution order shifts for GF and VX were observed relative to RI hydrocarbon standards when operating at faster LPTGC programming rates (thus at increasingly higher time-weighted average column temperatures).

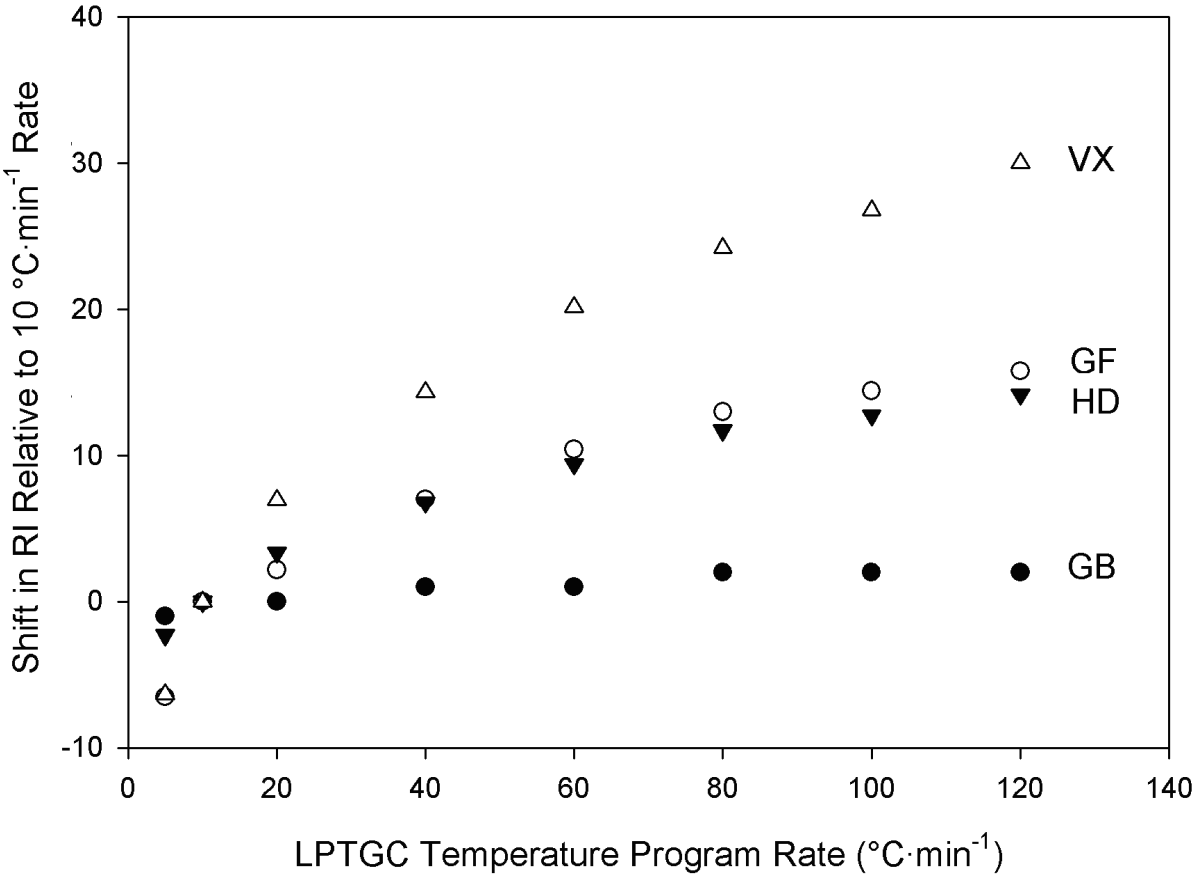


Figure 4.1. Shift in RI values as a function of LPTGC temperature program rate, relative to 10 $^{o}C \cdot min^{-1}$ heating rate for sarin (GB), sulfur mustard (HD), cyclohexyl methylphosphonofluoridate (GF), and O-ethyl S-2-diisopropyl aminoethylmethylphosphonothiolate (VX).

Table 4.2. Observed RI values for the analytes studied with varied He and H_2 carrier gas velocities (40, 60, 80, 100 and 120 $cm \cdot s^{-1}$); compounds were analyzed with column temperature at 40 ^oC for 30 s followed by LPTGC heating at 10 $^oC \cdot min$-1. Analyses were not possible using He carrier at 120 and H_2 at 40 $cm \cdot s^{-1}$ with the GC column used as controllable column head pressures could not support either combination.

		Carrier Gas Velocity ($cm \cdot s^{-1}$)				
		40	60	80	100	120
GB Reference	823.8[1]					
He		818.6±0.08	818.7±0.20	818.8±0.09	818.6±0.02	-
H_2		-	819.3±0.10	819.0±0.18	818.8±0.21	818.6±0.20
GD Reference [A]	1045.2[1]					
He		1039.6±0.06	1039.1±0.07	1038.8±0.14	1038.7±0.14	-
H_2		-	1039.4±0.04	1038.9±0.03	1038.7±0.07	1038.5±0.02
HD Reference	1172.7[1,2]					
He		1176.2±0.05	1174.1±0.12	1172.4±0.14	1170.9±0.21	-
H_2		-	1174.2±0.05	1172.4±0.05	1171.2±0.12	1170.2±0.10
GF Reference	1208[3]					
He		1205.5±0.003	1203.7±0.13	1202.5±0.07	1201.5±0.37	-
H_2		-	1203.9±0.07	1202.6±0.07	1201.8±0.14	1201.2±0.14
VX Reference	1710.1[1]					
He	1705.0[4]	1708.6±0.08	1703.9±0.09	1700.8±0.29	1698.5±0.47	-
H_2		-	1703.9±0.09	1701.0±0.18	1698.9±0.09	1697.2±0.19
$(DES)_2$ Reference	2057.94[4]					
He		2059.8±1.59	2053.5±1.03	2049.0±0.70	2046.8±0.75	-
H_2		-	2053.8±0.55	2049.1±0.43	2046.1±0.47	2043.9±0.44

[A]Based on retention time for 1st eluting peak of the GD enantiomeric pair; [1]D'Agostino *et al.* [21], [2]D'Agostino *et al.* [22], [3]Organization for the Prohibition of Chemical Weapons (OPCW) database [8], [4]D'Agostino *et al.* [13]. Standard deviation is noted in table. Coefficient of variation values calculated from replicate samples were <1% in all cases. Standard deviation did not exceed 0.90 for all compounds over the range of carrier gas velocity rates.

Table 4.3. Time-weighted average column temperature (from injection to elution) over the range of linear temperature programs used in the analysis of the earliest and latest eluting compounds studied (GB and $(DES)_2$ respectively); initial carrier gas linear velocity (H_2) of 100 $cm \cdot s^{-1}$, column temperature of 40 ^oC for 30 s followed by LPTGC heating at the rate specified. Coefficient of variation values calculated from replicate samples were <1% in all cases. Standard deviation values were below 0.30 in all cases.

Analyte	LPTGC Program Rate ($^oC \cdot min^{-1}$)	Time-weighted average Column Temperature (^oC)
GB	5	43.34
	10	45.86
	20	49.65
	40	55.06
	60	59.00
	80	64.15
	100	65.85
	120	69.80
$(DES)_2$	5	115.94
	10	124.79
	20	135.54
	40	145.98
	60	155.40
	80	160.66
	100	165.36
	120	170.72

Figures 4.2-4.4 show that HD, GF, and VX displayed elution order changes relative to reference hydrocarbons with higher isothermal analysis temperatures. Only GB and GD (figures not shown) did not display elution order changes with either higher isothermal temperatures or faster LPTGC ramping. The LPTGC elution order changes noted for GF and VX with faster temperature ramping were similar to retention characteristics observed under higher isothermal temperature conditions (Figures 4.3, 4.4, 4.5a, and 4.5b).

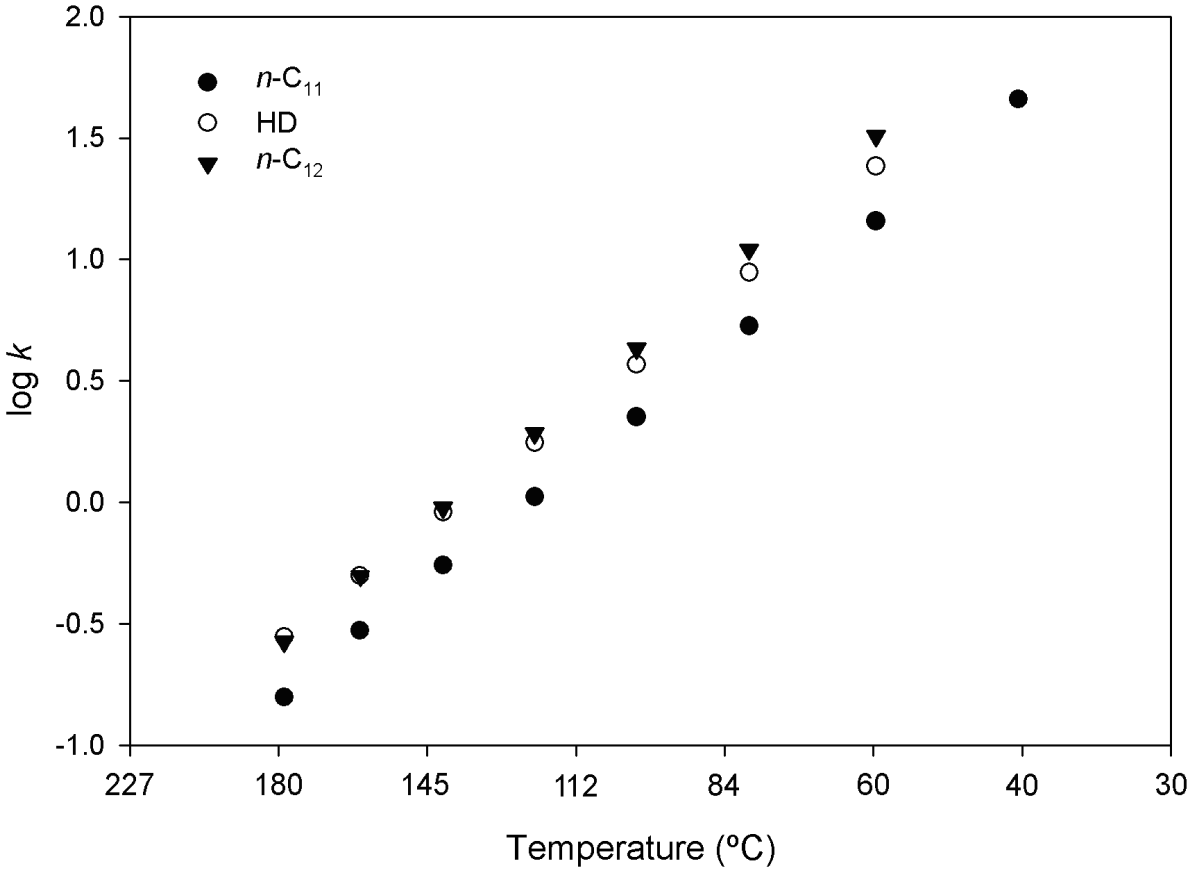


Figure 4.2. Retention factor (*k*) at varied isothermal analysis temperatures for the chemical warfare agent HD and its adjacent *n*-hydrocarbon reference compounds, *n*-C_{11} and *n*-C_{12}. To produce a straight line in Figures 4.2 through 4.4, the abscissa scale is the reciprocal of absolute temperature, labeled as °C to allow ready comparison to tabular data where this unit of temperature is used.

Isothermal conditions could be manipulated to produce shifts in elution order for HD relative to *n*-C_{12} (Figure 4.2) and with increasingly aggressive LPTGC ramping rates HD eluted closer to *n*-C_{12} as shown in Figure 4.5b. However the elution order for HD relative to *n*-C_{11} and *n*-C_{12} standards did not change with the LPTGC conditions studied.

The relatively small influence of LPTGC ramping rate on RI values for early eluting analytes such as GB and GD appears to be due to the high mobility of these analytes at the initial GC column temperature. This allows for column migration during the initial isothermal period of 40 °C used in all of the LPTGC analyses that provided RI

values found in Table 4.1. As a portion of the migration period for early eluting analytes was at the initial isothermal conditions, the use of faster LPTGC ramping rates only influenced the portion of the total column migration time which occurred during temperature ramping. Later eluting analytes either migrate more slowly, or not at all until the column temperature is higher, and thus either most or all of the migration time for such analytes occurs during temperature ramping, resulting in a proportionally greater time spent at higher column temperatures.

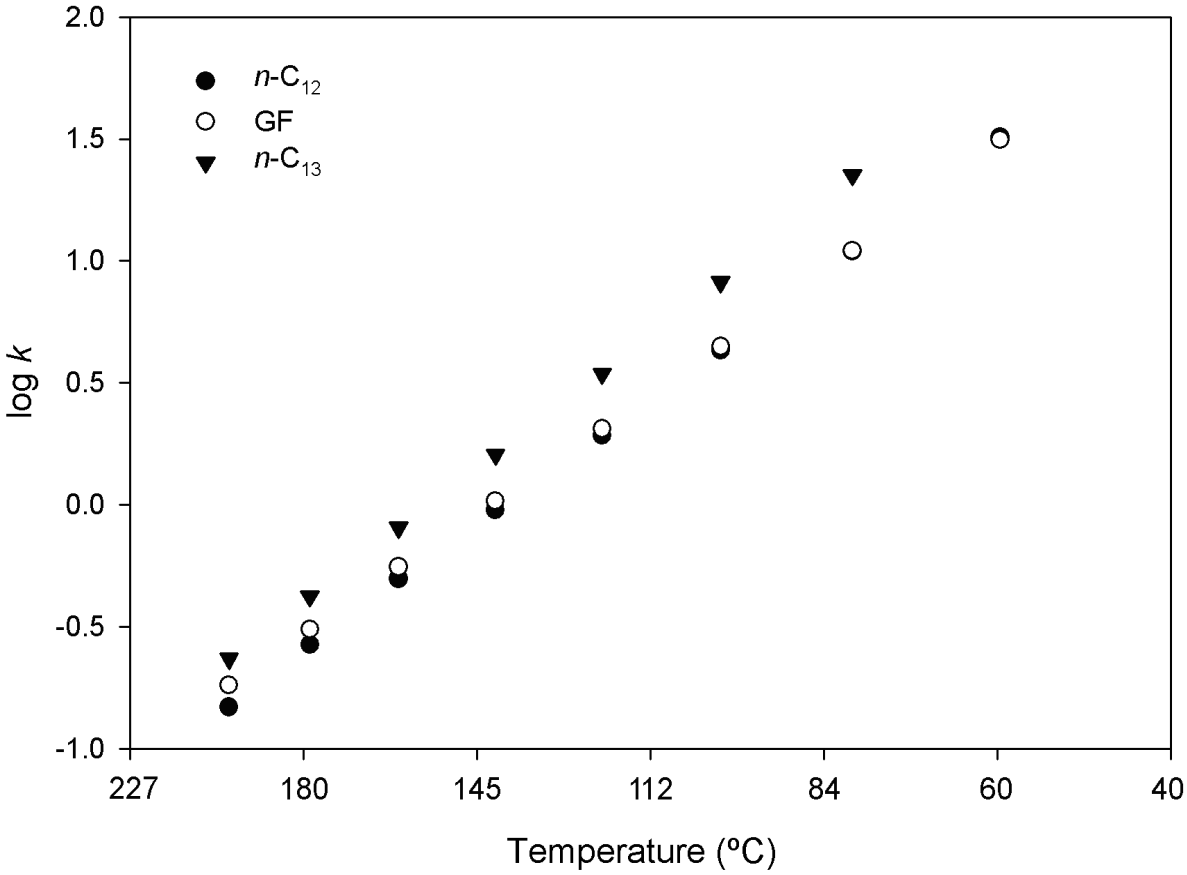


Figure 4.3. Retention factor (*k*) at varied isothermal analysis temperatures for the chemical warfare agent GF and its adjacent *n*-hydrocarbon reference compounds, *n*-C_{12} and *n*-C_{13}. For analysis at the lowest temperature, *n*-C_{13} did not produce a GC peak that was detectable by the data handling software used.

Table 4.4. Time-weighted average column temperature (from injection to elution) over the range of carrier gas linear velocities (60, 80, 100, 120 cm/s) used in the analysis of VX and $(DES)_2$; constant velocity as indicated; column temperature of 40 ^{o}C for 30 s followed by LPTGC heating at 10 $^{o}C \cdot min^{-1}$. Coefficient of variation values calculated from replicate samples were <1% in all cases.

Analyte	Carrier Gas Velocity (cm/s)	Time-weighted average Column Temperature (^{o}C)
VX	60	105.78
	80	101.70
	100	98.87
	120	96.37
$(DES)_2$	60	123.54
	80	119.37
	100	116.37
	120	113.78

By examination of Figures 4.2-4.4, the greatest change in retention with higher temperature occurs with the *n*-alkane standard compounds compared to the CWA-related study materials. The alkanes are initially more soluble in the stationary phase material and as isothermal temperature (or LPTGC ramping rate) rises the alkanes partition less to the stationary phase and the separation is based increasingly on volatility, and less on solubility in the stationary phase. The more polar CWA-related study compounds are relatively less affected by changes in temperature conditions, as they are much less soluble in the non-polar stationary phase from the beginning.

The work of Kokko *et al.* [5] showed that increased carrier gas flow rates gave lower RI values for later eluting CWA analytes, such as VX. This is consistent with our observations regarding the influence of time-weighted average column temperature between injection and elution on retention. Faster carrier gas linear velocity lessens the time analytes remain on the GC column, and this will lead to lower overall time-weighted

average column temperatures between injection and elution during LPTGC analyses compared to the use of slower linear velocity values, as shown in Table 4.4 for the relatively late-eluting analytes VX and $(DES)_2$. Data shown in Table 4.2 indicate that with constant and identical velocities the type of carrier gas used does not itself influence retention and cause RI value changes.

Figures 4.6a and 4.6b show that the time-weighted average LPTGC temperatures and corresponding values of k'_{LPTGC} for GB and GD fell close to the line formed by plotting k versus isothermal analysis temperature for these analytes.

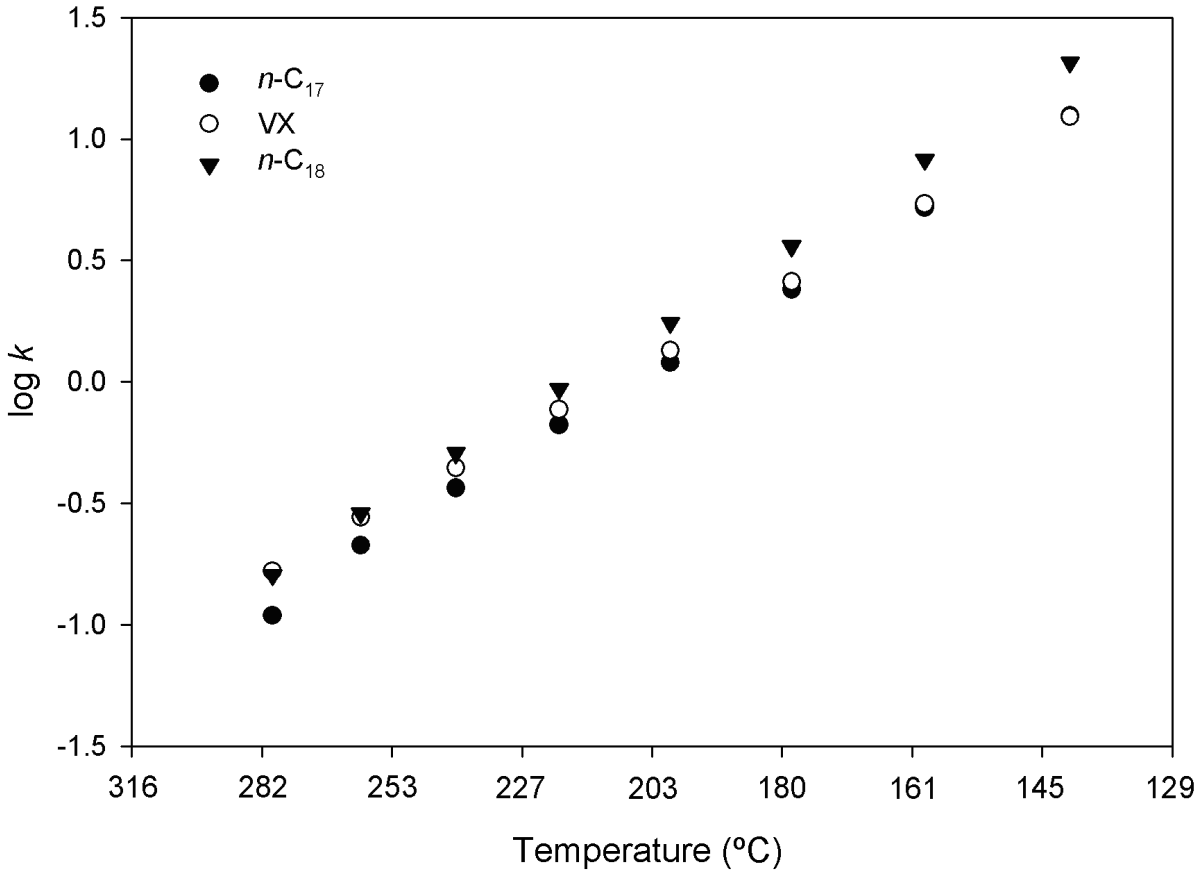


Figure 4.4. Retention factor (k) at varied isothermal analysis temperatures for the chemical warfare agent VX and its adjacent *n*-hydrocarbon reference compounds, *n*-C_{17} and *n*-C_{18}.

Figures 4.6c and 4.6d show that similar plots of k'_{LPTGC} values versus the time-weighted average column temperature for successively less volatile compounds studied were observed to increasingly diverge from the corresponding isothermal temperature and *k* value data sets. This observation underscores a limitation in using the time-weighted average column temperatures for the LPTGC programs to completely explain observed changes to RI values. As described by Giddings, a compound when first introduced onto a GC column may (depending on initial temperature conditions) undergo an initial "frozen" period, in which little movement occurs until a temperature is reached at which the partitioning from the liquid stationary phase into the carrier gas becomes significant [24]. As increasingly less volatile compounds tend to remain immobile at the head of the column for longer periods, using the entire range of temperatures between injection and elution in calculating time-weighted average GC column temperatures gives excess weight to portions of the analysis where there is little or no effective gas phase activity for a particular analyte. The effects of using the entire temperature range between injection and elution to calculate time-weighted average column temperature for comparison of k'_{LPTGC} values to values of *k* obtained under isothermal conditions can be visualized in Figures 4.6b and 4.6c, as plots for the higher molecular weight compounds (VX and $(DES)_2$) display increasingly greater gaps between the matched data sets.

A more relevant approximation of the time-weighted average temperature experienced by the chemicals in a linear temperature program would use only the period of time from the start of column migration to the time of elution in calculating the average column temperature. Figure 4.7b shows a much better match with k'_{LPTGC} values for $(DES)_2$ plotted against time-weighted average column temperature, compared to

values of *k* from isothermal analyses, when the initial column temperature allows for immediate or nearly-immediate gas phase activity of the analyte from the beginning of the LPTGC run.

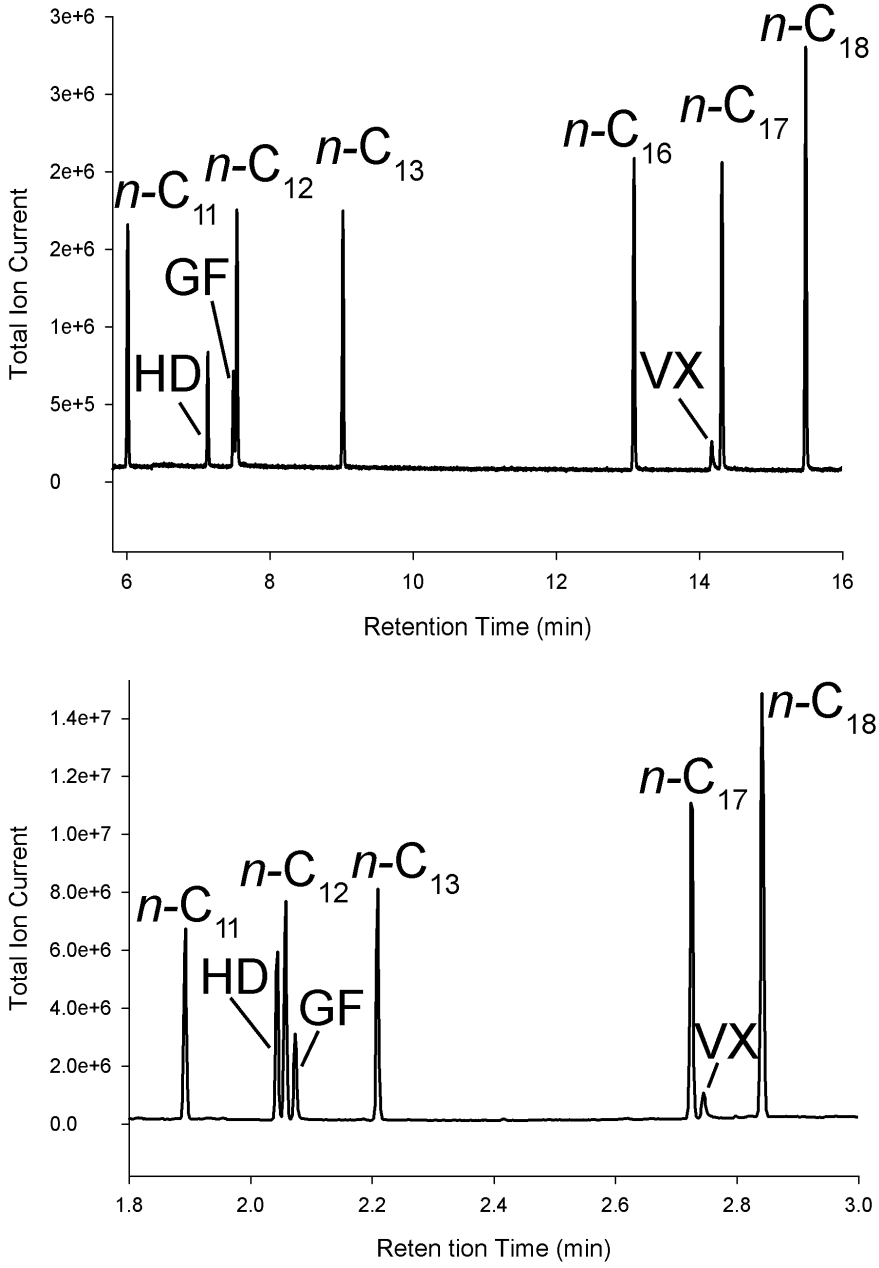


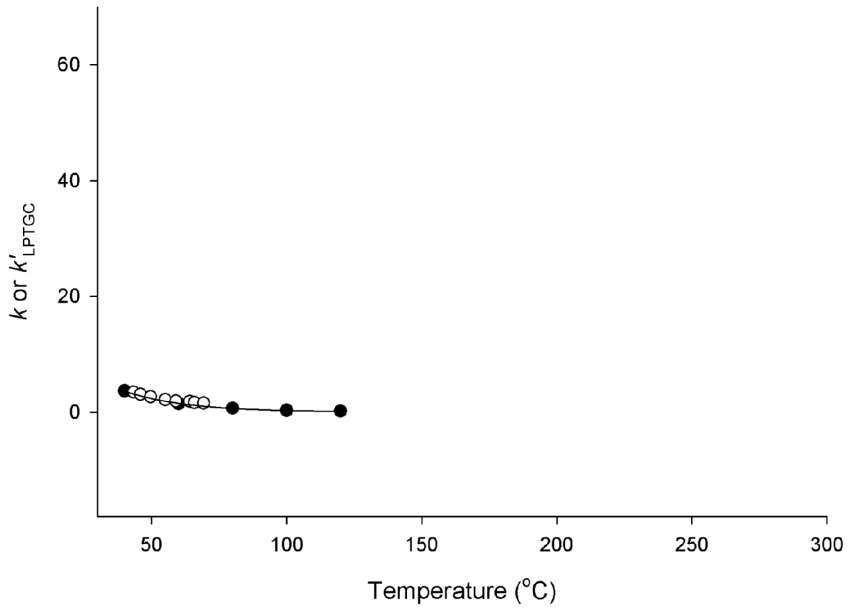
Figure 4.5a (top). GC/MS analysis of *n*-hydrocarbons and CWAs as noted with initial and terminal temperature of 40 (30 s hold time) and 300 °C and standard column heating rate of 10 °C·min^{-1}. **Figure 4.5b (bottom).** Liquid injection GC/MS analysis of the normal hydrocarbons and chemical warfare agent compounds as noted with identical initial and terminal temperature conditions but with fast heating rate of 120 °C·min^{-1}.

Table 4.5. Values of the distribution constant (*K*) for the analytes studied, based on retention characteristics and column phase ratio (β); isothermal analyses at the temperatures specified. Standard deviation and coefficient of variation values, respectively, are included in parentheses.

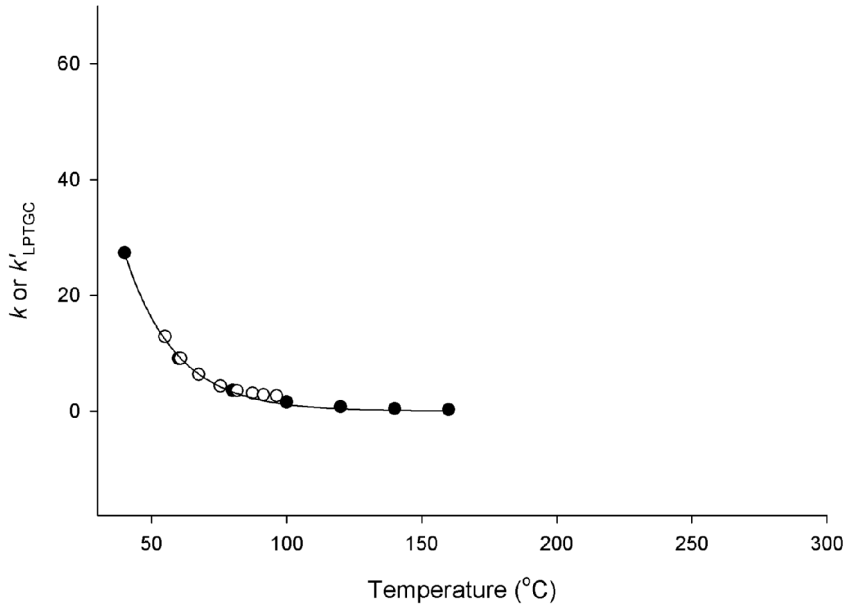
	GB	GD[a]	HD	GF	VX	$(DES)_2$
Temperature (°C)						
40	906 (2.12, 0.23)	6830 (5.50, 0.08)	-	-	-	-
60	367 (0.61, 0.17)	2278 (5.62, 0.25)	6050 (8.45, 0.14)	7860 (6.89, 0.09)	-	-
80	168 (0.44, 0.26)	891 (2.18, 0.24)	2214 (1.45, 0.07)	2753 (6.65, 0.24)	-	-
100	82 (0.45, 0. 55)	394 (1.22, 0.31)	926 (1.60, 0.17)	1114 (1.82, 0.16)	-	-
120	44 (2.63, 5.97)	196 (1.30, 0.66)	440 (1.77, 0.40)	513 (1.20, 0.23)	-	-
140	-	105 (0.34, 0.32)	228 (1.76, 0.77)	259 (1.21, 0.47)	3081 (3.60, 0.12)	-
160	-	59 (0.00, 0.00)	124 (0.38, 0.31)	139 (0.38, 0.28)	1349 (1.11, 0.08)	6770 (15.49,0.23)
180	-	-	70 (1.33, 1. 9)	77 (1.14, 1.48)	647 (2.89, 0.45)	2680 (1.50, 0.06)
200	-	-	-	45 (2.17, 4.82)	335 (1.80, 0.54)	1430 (4.75, 0.30)
220	-	-	-	-	190 (2.80, 1.47)	731 (9.69, 1.33)
240	-	-	-	-	110 (2.99, 2.72)	383 (1.08, 0.28)
260	-	-	-	-	69 (1.19, 1.72)	219 (1.03, 0.47)
280	-	-	-	-	42 (3.40, 8.10)	*

[a]based on retention time for 1st eluting peak of the GD enantiomeric pair. Dashed entries indicate that a GC peak that could be integrated by the software package used was not seen. The asterisk for $(DES)_2$ at 280 °C indicates that no sample was collected at this temperature.

A

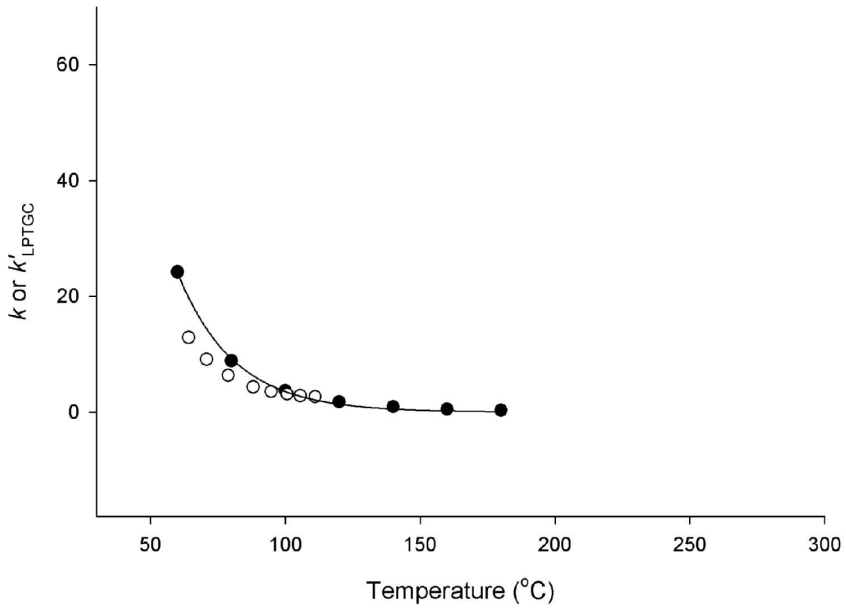


B

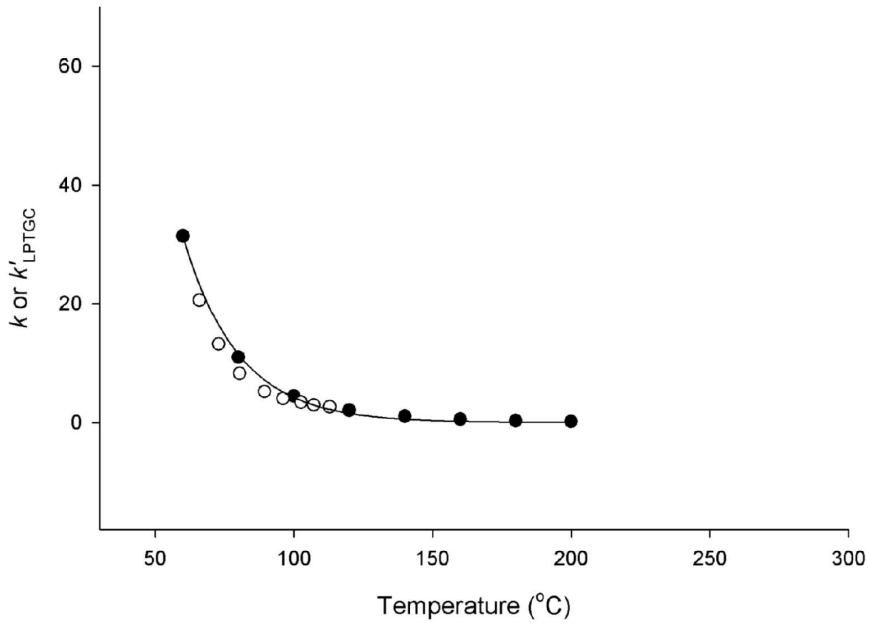


Figures 4.6a-4.6b. Retention factor (*k*) at isothermal analysis temperatures (dark icons) with start temperature of 40 °C for the chemical warfare agent **a,** GB and **b,** GD. The corresponding open icons in each graph show k'_{LPTGC} plotted against time-weighted average column temperatures between injection and elution.

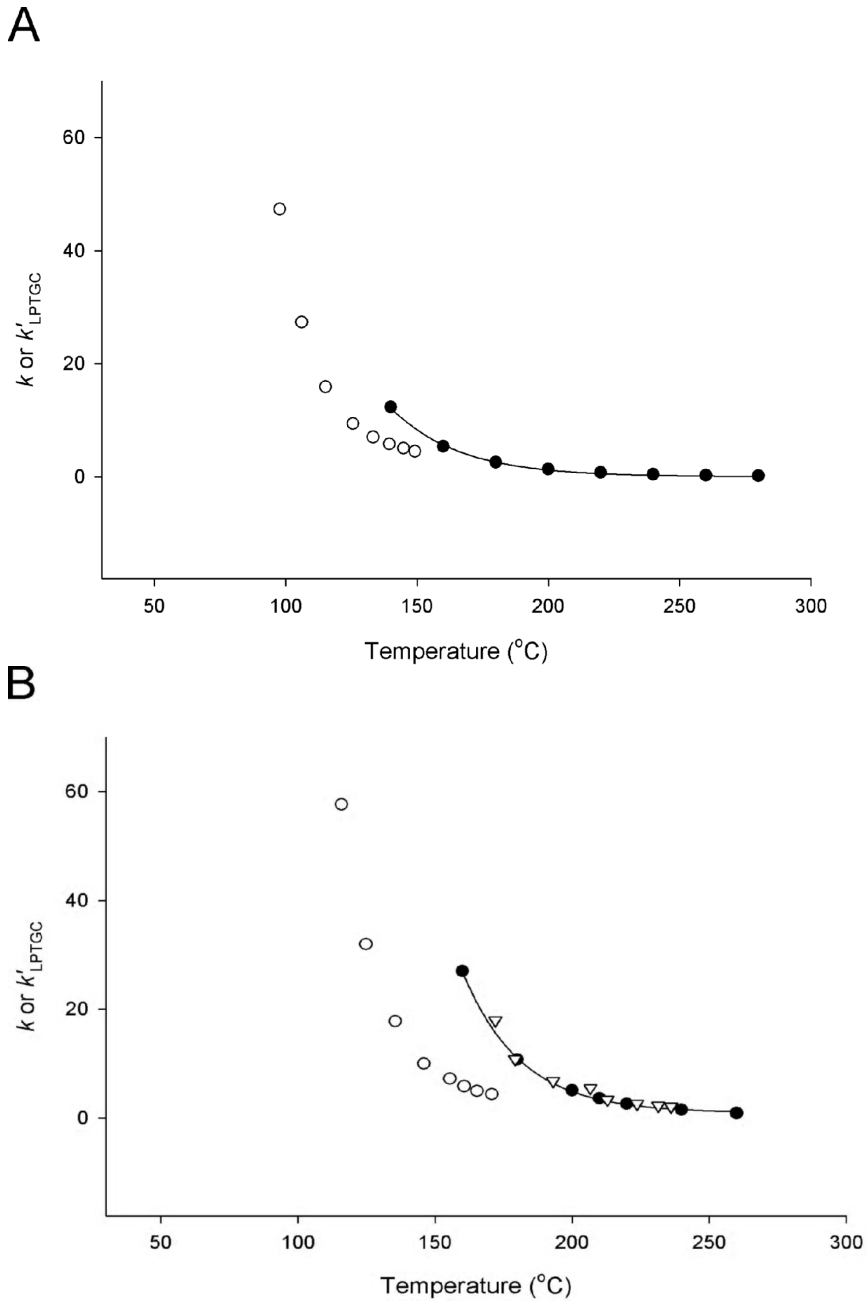
C



D



Figures 4.6c-4.6d. Retention factor (*k*) at isothermal analysis temperatures (dark icons) with start temperature of 40 °C for the chemical warfare agent **c,** HD and **d,** GF. The corresponding open icons in each graph show k'_{LPTGC} plotted against time-weighted average column temperatures between injection and elution.



Figures 4.7a-4.7b. a, Retention factor (*k*) at isothermal analysis temperatures (dark icons) with start temperature of 40 °C for the chemical warfare agent VX; the corresponding open icons show k'_{LPTGC} plotted against time-weighted average column temperatures between injection and elution. **b,** Retention factor (*k*) at isothermal analysis temperatures (dark icons) with start temperature of 40 °C for the VX degradation product $(DES)_2$; the corresponding open icons show k'_{LPTGC} plotted against time-weighted average column temperatures between injection and elution with 40 °C start temperature. The open triangular icons show k'_{LPTGC} plotted against the time-weighted average column temperatures between injection and elution for $(DES)_2$ with GC column start temperature of 150 °C.

Table 4.6. Observed and predicted values of k'_{LPTGC} for $(DES)_2$ with initial GC column temperature of 150 °C, no isothermal hold time (immediate temperature programming) at the rates indicated.

LPTGC Temperature Program Rate ($°C \cdot min^{-1}$)	Observed k'_{LPTGC}	Predicted[a] k'_{LPTGC}	$\Delta k'_{LPTGC}$	$\Delta k'_{LPTGC}$ (%)
5	18.0	14.7	3.3	18.3
10	10.9	10.4	0.5	4.6
20	6.9	6.8	0.1	1.4
40	5.5	4.1	1.4	25
60	3.4	3.0	0.6	18
80	2.6	2.4	0.2	7.7
100	2.3	1.9	0.4	17
120	2.1	1.7	0.4	19

[a]following the methodology of Podmaniczky *et al.* [19]

Table 4.5 provides calculated values of the distribution constant (*K*) for each chemical studied at the various relevant isothermal temperatures.

In the work of Podmaniczky *et al.* [19], calculated values for k'_{LPTGC} were compared to observed values for a range of LPTGC analytes with continual ramping at a single rate of either 2, 4, or 6 $°C \cdot min^{-1}$, and no initial isothermal hold segment. Using data produced from isothermal analyses of $(DES)_2$ in this work, predicted k'_{LPTGC} values calculated following the methods of Podmaniczky *et al.* were correlated with observed values for the same metric ($R^2 = 0.98$). On a percent basis, differences between predicted

and observed values ranged from negligible to 25% (Table 4.6). Similar data compiled by Podmaniczky *et al.* [19] using a much larger data set and LPTGC ramping rates $\leq$ 6 °C·min^{-1} showed differences between observed and predicted values for k'_{LPTGC} ranged from negligible to 17%.

The connection between column temperature conditions and potential changes to RI values is of use to others beyond the audience interested in fast GC for analysis of highly dangerous chemicals. Jonsson *et al.* [25] note the potential usefulness of RI data from GC/MS analyses taken in combination with mass spectra for automated metabolomics investigations. This and other evolving fields could benefit from the rapid analysis times made possible by fast temperature programming based on modern column heating approaches. In order for researchers in many disciplines to take advantage of RI data derived from such analysis conditions, the influence of rapid column heating on the resulting RI values must be accounted for.

4.4 Conclusion

Isothermal analyses at varied temperatures showed the relationship between the values of *k* for each CWA-related analyte, to the value of *k* for the respective reference hydrocarbons. In the cases of CWA analytes with the largest relative RI value shifts under rapid temperature programming conditions (VX, GF, and HD), changes to isothermal analysis temperatures showed the greatest effect on *k* relative to the respective reference hydrocarbons, and even elution order changes were shown to be possible with rapid LPTGC ramping. The shifts in RI values for the compounds studied with rapid LPTGC ramping are similar to those observed under higher temperature isothermal

conditions. This is explained by examining the relationship that exists between LPTGC ramping rate and time-weighted average column temperature between injection and elution.

A faster temperature programming rate provides for higher elution temperatures and time-weighted average GC column temperatures than slower programming rates. When k'_{LPTGC} is plotted against the time-weighted average temperature from various LPTGC ramp rates, a reasonable fit is observed compared to k plotted against isothermal analysis temperatures, but only for early eluting compounds or for analysis conditions where the starting GC column temperature will provide immediate gas phase activity. Any factor (such as carrier gas linear velocity) that affects the time-weighted average column temperature between injection and elution will have the potential to change RI values.

Knowledge of the factors that influence expected RI values during LPTGC with rapid temperature ramping rates is important as high performance laboratory and field-portable GC instruments become available with fast GC temperature ramping and correspondingly rapid analysis times. The usefulness of existing RI database values collected under standard conditions with ramping at 10 $^{o}C \cdot min^{-1}$ can be extended to these conditions with some additional work.

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Chapter 5

Conclusion/Future Studies

The goal of this research was to examine potentially improved sampling and separation/analysis methods and techniques in the identification of trace-level organic compounds of interest. The sampling and separation/analysis methods employed in this research were intended to answer the questions initially posed. Specifically, "can we collect enough mass of a chemical warfare-like compound in only a matter of seconds to provide for definitive detection at ppb_v and ppt_v concentrations?" and "can we provide predictable, definitive, on-site analytical results for chemical warfare agents (CWA) even when performing the separation and subsequent analysis of these compounds in significantly faster time relative to standard operating conditions?" To answer these questions, two primary focus areas were researched: 1) a newly developed dynamic sampling technique was used to collect CWA surrogate and degradation compounds, and 2) chemical warfare agent retention characteristics were determined when analysis used fast GC methods relative to the standard 10 $^{o}C \cdot min^{-1}$ temperature ramp rate.

The HSA-SPME air sampling device discussed in this work is the product of several years of collaborative research effort, with a particular focus on improving the speed and sensitivity of trace-level VOC detection capabilities for personnel operating in contaminated environments. The analytical methods used in this research permit rapid sampling and fast analysis techniques. In the field, fast analysis is of equal importance to rapid sampling in delivering effective intelligence quickly to the on-scene commander, which underscored the reason for studying potential improvements in both the sampling and analysis approaches. The operators whom this research supports are primarily: 1)

military chemical detection response units such as the Chemical and Biological Incident Response Force (CBIRF) of the U.S. Marine Corps, as well as the U.S. Army 20th Support Command's Technical Escort Unit, and National Guard Civil Support Teams (CSTs), 2) civilian emergency response teams, to include fire and rescue and HAZMAT teams, as well as 3) special law enforcement units, particularly those working in the arenas of forensics research and criminal investigations.

The desired field sampling method is easy-to-use, rapid, sensitive, and capable of selective collection of multiple compounds with different physical and chemical characteristics. Use of the HSA-SPME air sampling method represents a potential step forward in meeting these needs, when compared to other currently utilized field methods such as PID, IMS or passive SPME sampling techniques. An approximately 50% increase in analytical response relative to traditional means of VOC sorbent collection was observed with HSA-SPME device when sampling for brief periods ranging from 5 to 30 s. Even greater improvements in sensitivity were observed when using tandem HSA-SPME, as detection in the parts per trillion range for CWA-related compounds was possible (with extracted ion chromatograms) when sampling for 10 s.

However, while HSA-SPME provides definite advantages, further improvements in sampling capabilities are possible. The arena of chemical collection and detection research is growing rapidly. Improvements to HSA-SPME sampling have already been postulated and collaborative discussions for fabricating the next generation of the dynamic SPME chemical detection device are ongoing at the FBI's Counter-Terrorism Forensic Science Research Unit (CFSRU). The ultra HSA-SPME method (uHSA-SPME), the latest notional iteration of active SPME sampling [1], is currently in the

conceptual phase. The idea involves additional increases in sorbent surface area while also improving desorption and delivery of trapped chemical compounds from the sorbent into an analytical instrument.

The applications for an uHSA-SPME sampling device design following this approach are wide-ranging. One example would be a miniature sampling device weighing only a few grams that could be clipped to the shirt pocket of a worker for the period of a day or longer to determine occupational exposure levels for various chemical hazards in the workplace. Additional applications could include short or long-term exposure monitoring for CWAs, TICs and other volatiles in areas of military or law enforcement importance.

An additional improvement to HSA-SPME field use could involve the optimization of its pre-concentration process. A fieldable, miniaturized pre-concentrator system designed specifically for a ruggedized GC/MS system like the LTM GC is currently under development and testing. This microtrap design has been successfully used in conjunction with a laboratory-based LTM GC system [2,3]. However, improved filed-portable focusing pre-concentrator designs are needed, and should be ruggedized and made to be user-friendly.

The utility of fast GC programming could not be fully realized without characterizing the impact of fast GC rates on the retention characteristics of the sampled chemicals of interest. The retention characteristics under linear temperature programming were found to be reproducible over a wide range of fast GC ramp rates, based on use of time-weighted-average (TWA) column temperatures and their effect on the retention factor. This research provides valuable knowledge to those employing

faster GC ramp rates in an effort to provide definitive answers rapidly to incident commanders, as it provides confidence in the use of retention index (RI) information derived for the chemicals of interest, no matter the ramp rate used.

The RI research discussed in Chapter 4 explained the retention behavior of 5 chemical warfare agents (CWAs) and one CWA degradation product while subjected to a range of isothermal and linear temperature programs. This research demonstrated that a faster linear temperature ramp rate mathematically leads to an increased TWA column temperature over a range of ramp rates from the point of injection to elution. Earlier eluting compounds display mobility in the gas phase upon introduction onto a column heated at initial column temperatures of 40 ºC to 60 ºC, and thus their corresponding k'_{LPTGC} values, which refers to the retention factor (*k)* under linear programmed temperature gas chromatography (LPTGC) conditions, correlate well with *k* values from isothermal temperatures matching the TWA temperature of the LPTGC run. For later eluting compounds, a simple TWA temperature was found to insufficiently explain the compound's retention behavior, as a fair portion of the TWA temperature was observed to include a period of immobility of the compound on the stationary phase of the GC column. It was found that a more accurate mathematical description of the relationship between column temperature and k'_{LPTGC} values for later eluting compounds was provided when calculating the TWA temperature beginning at the point when the respective chemical compound has appreciable gas phase activity until it elutes from the GC column.

Additional observations included elution shifts at hotter column temperatures for both isothermal and LPTGC programs. It was observed that a change in elution order

between an analyte of interest and its neighboring *n*-hydrocarbons occurred at higher isothermal temperatures, and this same phenomenon occurred with linear programmed temperature ramps with a correlated TWA temperature to the isothermal temperature. These shifts were found to be reproducible over a range of isothermal and linear temperature profiles and occurred at similar temperature points when comparing the isothermal retention factor values obtained under isothermal temperature programs to the retention behavior of the CWA compounds at the correlated TWA temperatures calculated for the linear temperature programs.

In conclusion, HSA-SPME devices combined with rapid GC analysis demonstrate potential benefits to field operators in hazardous work environments. On-scene commanders are first and foremost concerned with the safety and health of the public and the responders under their command; reducing the time required in the "hot zone", which the HSA-SPME device provides along with good sensitivity, is certainly consistent with adhering to this primary concern. Additionally, the ability to provide a definitive response for a harmful chemical at the ppt_v level offers a greater level of safety for the casualties and providers, as well as increased confidence for emergency response personnel that their casualty decontamination was sufficient prior to moving casualties into a treatment facility. Law enforcement personnel at the scene could be provided with a sampling device sensitive enough to gather chemical clues which would further assist in directing their investigative efforts.

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