3-24-2017

Evaluation of Cryofocusing Capillary Microextraction of Volatiles for Improved Detection of Organic Gunshot Residue on the Hands of Shooters

Jerome Mulloor
Florida International University, jmull008@fiu.edu

DOI: 10.25148/etd.FIDC001776

FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

EVALUATION OF CRYOFOCUSING CAPILLARY MICROEXTRACTION OF VOLATILES FOR IMPROVED DETECTION OF ORGANIC GUNSHOT RESIDUE ON THE HANDS OF SHOOTERS

A thesis submitted in partial fulfillment of

the requirements for the degree of

MASTER OF SCIENCE

in

FORENSIC SCIENCE

by

Jerome Mulloor

2017

To: Dean Michael R. Heithaus
College of Arts, Sciences and Education

This thesis, written by Jerome Mulloor, and entitled Evaluation of Cryofocusing Capillary Microextraction of Volatiles for Improved Detection of Organic Gunshot Residue on the Hands of Shooters, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

_____
Anthony DeCaprio

_____
DeEtta Mills

_____
Yi Xiao, Co-Major Professor

_____
José Almirall, Co-Major Professor

Date of Defense: March 24, 2017

The thesis of Jerome Mulloor is approved.

_____
Dean Michael R. Heithaus
College of Arts, Sciences and Education

_____
Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2017

DEDICATION

I dedicate this work to my family and friends who supported me unconditionally throughout my graduate career and helped me reach the finish line.

ACKNOWLEDGMENTS

First, I would like to thank my advisor, Dr. José Almirall, for granting me the opportunity to work in his lab. The knowledge I gained as a student and the experience I gained as a researcher under his guidance were profoundly influential in my graduate career. I also thank Dr. Yi Xiao since her passion for research inspired me to aim for excellence. I am thankful for Dr. Anthony DeCaprio's support with this project as well as the knowledge and presentation skills I gained in his classroom. To Dr. DeEtta Mills, I am very grateful to her for assisting me and devoting time from her demanding schedule.

I consider myself lucky to be a part of the "A-team". Thanks to my labmates, friends, and colleagues for their support and making me feel like part of a family. I am forever grateful that I had the chance to work with Dr. Sigalit Gura, who provided gentle guidance during the project. Special thanks to Dr. Anamary Tarifa for her advice and always lending a helping hand. Thanks to Dr. Luis Arroyo and Dr. Tatiana Trejos and the rest of the IFRI staff, especially our "lab mom" Pupi Tomassini, for all their hard work.

I also thank Officer Jose Ramirez of the FIU police department and Chief Alexander Casas for supporting our research efforts. Thanks to Gabriel Hernandez of the Miami-Dade Police Department for allowing me to take his workshop on firearms which was a valuable hands-on learning experience. I acknowledge Dr. Norman Huang for his assistance with building the Peltier cooler and our lab neighbor Zahid Baccus for his help from the machine shop.

Finally, I would like to acknowledge RTI for providing funding and assistantship for this project.

ABSTRACT OF THE THESIS

EVALUATION OF CRYOFOCUSING CAPILLARY MICROEXTRACTION OF VOLATILES FOR IMPROVED DETECTION OF ORGANIC GUNSHOT RESIDUE ON THE HANDS OF SHOOTERS

by

Jerome Mulloor

Florida International University, 2017

Miami, Florida

Professor José Almirall, Major Professor

The capillary microextraction of volatiles (CMV) device was equipped with a novel Peltier cooler to investigate cryofocused extraction of organic gunshot residue (OGSR) for the first time. Prior research demonstrated the CMV's capabilities for detecting nitroglycerin, 2,4-dinitrotoluene, diphenylamine, and ethyl centralite on shooters' hands via gas chromatography-mass spectrometry. Further method development increased the recoveries of these four target compounds with an optimal 20-minute equilibrium time at 80˚C followed by extracting 3 L at a 1 L/min flow rate. The Cryo-CMV was evaluated for detection of semi-volatile OGSR compounds. The unique challenges presented with sampling of semi-volatiles were overcome by sample heating, applying high (>1 L/min) sampling flow rates and heating the transfer line between the container and cooled CMV. Cryofocusing at -10˚C provided increased recoveries for smokeless powders and OGSR compounds and therefore demonstrates excellent potential for other forensic applications with analysis of VOCs from fire debris and illicit drugs.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE PAGE

LIST OF ABBREVIATIONS AND ACRONYMS

2,4-DNT	2,4-Dinitrotoluene
ASTM	American Society for Testing Materials
BTEX	Benzene, toluene, ethylbenzene, xylene
CE	Capillary Electrophoresis
CMV	Capillary Microextraction of Volatiles
DC	Direct current
DI	Deionized
DPA	Diphenylamine
EC	Ethyl Centralite
ECD	Electron Capture Detector
EDX	Energy Dispersive X-ray
EI	Electron impact
eV	Electron volts
FBI	Federal Bureau of Investigation
FID	Flame ionization detector
FIU	Florida International University
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GSR	Gunshot Residue
HPLC	High performance liquid chromatography
ID	Internal diameter
IGSR	Inorganic Gunshot Residue

IMS	Ion Mobility Spectrometry
kPa	kilopascals
LC	Liquid Chromatography
LOD	Limit of detection
m/z	Mass-to-charge
MS	Mass Spectrometry
ng	Nanogram
NG	Nitroglycerin
OGSR	Organic Gunshot Residue
OSAC	Organization of Scientific Area Committees
PAH	Polycyclic aromatic hydrocarbons
PDMS	Polydimethylsiloxane
PFTE	Polytetrafluoroethylene (Teflon)
ppm	Parts per million
PSPME	Planar solid phase microextraction
SEM	Scanning Electron Microscopy
SIM	Selective ion monitoring
SNR	Signal to noise ratio
SPE	Solid phase extraction
SPME	Solid Phase Microextraction
SVOC	Semi-Volatile Organic Compound
TEA	Thermal energy analysis
TIC	Total ion current

TSP	Thermal Separation Probe
U.S.	United States
UPLC	Ultra-performance liquid chromatography
VOC	Volatile Organic Compound

1. INTRODUCTION

Firearm-related incidents, which include armed robberies, school shootings, and suspected suicides, require the utmost attention of law enforcement. The latest data from the Federal Bureau of Investigation (FBI) Uniform Crime Reports indicate there were 9,616 murder victims by firearm in the United States in 2015, which is an increase from 2014. [1] The Bureau of Justice Statistics National Crime Victimization Survey shows there were 466,113 victims of non-fatal violent crime involving a firearm in the United States in 2014. [2]

Forensic scientists must determine who was responsible for discharging the firearm in these cases. Rapid identification and apprehension of the suspected shooter is critical because of the severe nature of the crime and the imminent threat to the public. Police officers and crime scene investigators require a reliable, consistent, and rapid technique to collect the appropriate evidence in the field that enables forensic scientists to reach accurate conclusions in the laboratory.

When a firearm is discharged, various particles originating from the chemical components in the ammunition are deposited on the hands and clothing of the shooter, which is known as gunshot residue (GSR). Forensic scientists investigating firearm-related incidents rely on GSR as the primary form of evidence. Gunshot residue is useful for distance determination, identifying bullet holes, and determining if an individual handled a firearm. [3] Therefore, establishing verified and dependable methods for collection, extraction, and analysis of GSR is crucial.

1.1. Research Motivation

Techniques to identify individuals who have discharged a firearm are valuable to law enforcement, crime scene investigators, and forensic scientists. There are a variety of reported methods for sampling, extraction, and analysis of both inorganic and organic GSR, each with its own benefits and drawbacks. Gunshot residue is recoverable by sampling of the hands, hair, or clothing. [3] Collection methods include tape lifting, vacuuming, dabbing with carbon adhesive, and swabbing with various solvents. [3] Several presumptive color spot tests are available for nitrites [4] and lead to estimate firing distance and quickly assess the presence of GSR, but they are susceptible to false positives. Instrumental analysis techniques reported for GSR include liquid chromatography (LC), gas chromatography (GC), mass spectrometry (MS), ion mobility spectrometry (IMS), and capillary electrophoresis (CE). [5]

Currently, the most widely employed confirmatory technique is scanning electron microscopy with energy dispersive X-ray detector (SEM/EDX) as outlined in the ASTM E1588 guidelines. [6] In order to collect GSR, a carbon adhesive stub is dabbed on the hands of the suspect. The surface of the stub is analyzed using the instrument to identify GSR particles by their morphology and elemental composition. The characteristic elements in GSR are lead, barium, and antimony, which originate mainly from the primer in the cartridge. [6]

Although it is a fundamentally valid technique, SEM/EDX has disadvantages with regard to IGSR analysis. The instrumentation is not portable or suitable for fast field sampling. The analysis time can take several hours, which is undesirable when a quick determination of the presence of GSR on a shooter's hands is necessary. Moreover, there

is a legislative movement worldwide to remove lead from ammunition because of its toxicity and negative environmental effects. The changes will significantly affect the elemental data which SEM/EDX relies on to characterize GSR; the absence of lead will reduce the weight of the sample evidence. False negatives that result from the lack of lead or false positives because of a similar composition with brake dust may introduce problems with the evidence interpretation. [7]

Therefore, there is a need for a technique that can analyze organic gunshot residue alongside inorganic gunshot residue to address these concerns. A relevant field sampling technique for GSR on the hands of shooters will emphasize simple collection and rapid analysis. Furthermore, the technique should appeal to forensic laboratories because of its low costs and accommodation of already available equipment and instrumentation.

The current research is centered around the capabilities of an innovative capillary microextraction of volatiles (CMV) device invented, designed, and manufactured in Dr. José Almirall's laboratory at Florida International University. The CMV is a sorbent tube that can capture volatile compounds in the air and retain them for subsequent analysis using gas chromatography-mass spectrometry (GC-MS). The CMV is capable of qualitative and quantitative analysis of a wide range of volatile compounds, including explosives, ignitable liquid residues, headspace signatures of marijuana, and organic gunshot residue.

Previous research on the CMV device illustrates the benefits it provides for analysis of volatiles. Dynamic headspace extraction with the CMV avoids tedious sample preparation associated with other liquid extraction techniques. The CMV offers greatly reduced extraction times and higher sensitivity compared to a solid phase microextraction (SPME) fiber for similar applications with volatiles. [8] The CMV devices are an excellent

choice for field sampling because they can attach to portable air sampling pumps to draw in and capture volatiles. The CMV can be sealed and stored for several days to retain the sampled analytes. Once returned to the lab, they can be directly inserted into the injection port of a GC-MS via a commercially available adapter for rapid analysis. Characteristic compounds are subsequently identified and may yield significant findings regarding what was originally sampled.

Prior studies demonstrated the CMV's applications for OGSR analysis. [9] Gunshot residue contains volatile organic compounds (VOCs) that can be sampled and analyzed using the CMV device. Previous research by Tarifa and Almirall demonstrated the utility of the CMV for headspace extraction of hand swab samples from individuals who fired a gun. [9] The CMV method was capable of detecting VOCs on the hands of shooters via GC-MS, a common instrument in forensic laboratories for its utility as a confirmatory technique.

In the current research project, a novel concept of cryofocusing, in which the CMV is cooled during the extraction, was tested to potentially improve its extraction capabilities for volatiles in GSR. A custom-built Peltier cooler designed to accommodate the CMV was evaluated and optimized for this purpose. Cryofocusing-CMV-GC-MS showed improvements in recoveries when applied to extraction of toxic VOCs in indoor air. [10] Cryofocusing is expected to provide enhanced recoveries for extraction of the OGSR volatile compounds as well.

Therefore, there are two main aims of this research which revolve around the collection, extraction, and detection of organic GSR on the hands of shooters. First, the previously demonstrated CMV-GC-MS technique for OGSR analysis is further optimized.

Second, cryofocusing of the CMV is evaluated to determine whether it will provide benefits for the extraction of these compounds. Ultimately, these improvements are aimed at enhancing this method for practicality and implementation in crime laboratories.

1.2. Significance of Study

A forensically relevant method for organic GSR that complements inorganic GSR analysis will provide additional information to increase the value of the evidence. An expected increase in lead-free ammunition will complicate SEM-EDX analysis for inorganic GSR, which relies on lead as a characteristic element. Currently, there is no universal collection, extraction, and analysis method for organic GSR. [5] The Organization of Scientific Area Committees (OSAC) stresses the need for a comprehensive OGSR analytical method. [11] Therefore, improving the capabilities of the CMV-based method for collection and extraction of organic GSR will be valuable. The application of cryofocusing and method optimization are expected to provide further benefits to an already promising technique.

The CMV device holds significant potential for application in forensics. Its low cost and simplicity are appealing for crime laboratories. Additionally, implementation of the CMV is practical since it utilizes a commercially available adapter designed to fit on existing GC-MS instrumentation. Therefore, since the CMV possesses many desirable qualities that can address the issues in GSR analysis, further research into this technique is valuable to the forensic science community.

The evaluation of cryofocusing CMV-GC-MS (Cryo-CMV-GC-MS) for OGSR extraction is also significant for its extension into other areas of forensic science. The CMV holds incredible potential for rapid analysis of volatile compounds. Further knowledge of

cryofocusing of the CMV may provide useful insight for analysis of ignitable liquid residues from fire debris samples, headspace signatures of drugs, and air quality monitoring.

2. BACKGROUND AND THEORY

2.1. Formation of Gunshot Residue

A brief insight into the mechanism of operation of a firearm is essential to fully understand how gunshot residue (GSR) forms. First, the shooter pulls the trigger on a firearm which releases the hammer under spring tension. The hammer contains a firing pin that subsequently strikes the primer at the back of the cartridge with great force. The primer is shock sensitive and detonates as a result of the impact, sending a spark to the gunpowder in the cartridge. [12] The gunpowder ignites and burns within a fraction of a second. The ensuing chemical reactions cause gases to rapidly build up in the chamber resulting in very high pressure (10^4 kPa) and temperature (3600 °C). [13] The high pressure propels the bullet forward and out the muzzle at high velocity. Along with the bullet, the vapors from the burning of the gunpowder and primer exit the gun as well. These heated combustion products rapidly recondense upon encountering ambient temperature and pressure to form droplets of gunshot residue. [12] The resulting vapors and particulates are spread across the immediate vicinity and deposited on the shooter.

The components of GSR are typically classified as organic (OGSR) and inorganic (IGSR). Inorganic components originate from the primer, cartridge case, and weapon barrel; they typically consist of nitrates and metallic particles. [3] Organic components originate from the propellant and gunpowder additives; these contain breakdown products and hydrocarbons. [3] Both components are constituents of the fine particles and droplets scattered following the discharge of a firearm.

2.2. Evidentiary Value of GSR

Forensic scientists require reliable evidence that links a suspected shooter to the discharge of a firearm. Gunshot residue is a valuable form of evidence in firearm-related cases with several applications. [3] Bullets and spent cartridges are the link between the shooting and a specific gun. Similarly, gunshot residue is the link between the shooting and the individuals involved.

Crime laboratories currently utilize GSR to estimate muzzle-to-target shooting distance and identify bullet holes on garments. Sections of clothing submitted to the laboratory are processed with a series of chemical screening tests. The Modified Griess Test is a chromophoric reaction specific for nitrite residues deposited on the clothing from the burnt smokeless powder. An estimated firing distance range is obtained according to the size and diameter of orange patterns indicating a positive result for GSR. [4] The sodium rhodizonate test is another color-producing chemical test specific for lead residues. A positive reaction surrounding a hole on a garment indicates the passage of a bullet. [14] Although they are effective and established techniques, both tests require numerous reagents and extensive sample preparation.

Investigators also link a suspect or victim to the firing of a gun by identifying the presence of GSR on the hands or clothing. There are numerous methods for collection of GSR from a suspected shooter. Detecting GSR on the hands indicates that the person held or was in the vicinity of a discharged firearm. An analytical technique that evaluates the presence of GSR on a suspect’s hands is essential since photo or video evidence is not always available.

2.3. Inorganic Gunshot Residue

2.3.1. Components of Inorganic Gunshot Residue

The main chemical components of IGSR include metals, nitrates, and nitrites. These primarily come from the primer, bullet, cartridge casing, and barrel of the firearm. [15] One of the main sources of IGSR is the primer. The modern Sinoxid primer formulation consists mostly of lead styphnate, barium nitrate, and antimony trisulfide. [16] The high temperatures following the primer detonation exceed the melting points of lead, barium, and antimony, resulting in vaporized particles that eventually condense into droplets at ambient conditions. [3] Various nitrates, such as lead nitrate, sodium nitrate, strontium nitrate, and potassium nitrate are found in additives to the propellant powder and primer. [3]

2.3.2. Collection of Inorganic Gunshot Residue

Collection techniques for IGSR vary and depend on how sample analysis is performed in the laboratory. Scanning electron microscopy (SEM) with energy dispersive X-ray detector (EDX) is the most common analysis technique for IGSR and collection techniques are designed to accommodate it. The predominant collection technique for IGSR is lifting with an adhesive. [17] Typically, an aluminum stub with coated adhesive is pressed several times on the hands. Tape lifting demonstrates greater effectiveness than swabbing for SEM/EDX analysis. [3]

2.3.3. SEM/EDX Analysis of IGSR

The standard analysis technique employed by forensic laboratories for IGSR analysis is scanning electron microscopy with energy dispersive X-ray detector

(SEM/EDX). [3] Prior to the implementation of SEM/EDX, several bulk elemental techniques were used to analyze IGSR, such as neutron activation analysis, atomic absorption spectrometry, and inductively coupled plasma. [18] The main drawback to these techniques was their inability to provide conclusive results since many of the elements in IGSR are also found in the environment. [18] Another disadvantage to bulk analysis techniques is their destructive nature.

High resolution imaging of particles is achieved with the scanning electron microscope. Instead of light, SEM focuses a beam of electrons on the sample, providing higher resolution and magnification (100,000 X). [13] An X-ray detector enables analysis of the elemental composition of the sample. Electrons that strike the sample cause emission of X-rays at wavelengths characteristic to each element. [13] Thus, SEM/EDX can simultaneously image the GSR particles and identify the elements in them.

Nesbitt was one of the first to apply SEM/EDX for IGSR analysis on the hands. [19] Wolten extensively studied SEM/EDX in 1979 for IGSR analysis in forensic casework. First, he evaluated hand swabs from several different types of handgun cartridges and demonstrated the applicability of the technique for casework. [18] Next, he identified potential sources of GSR-like particles encountered in the environment or in certain occupations. [20] Basu categorized IGSR particles into three categories according to their frequency of detection and mechanism of formation. [21]

The many advantages of SEM/EDX led to its widespread acceptance as the standard IGSR analysis technique. Since it is non-destructive, it allows for morphological analysis with the capability of imaging individual particles. The American Society for

Testing and Materials (ASTM) E1588-10 standard outlines the criteria for sample processing and evaluation. Figure 1 shows a typical SEM image and accompanying EDX spectra. GSR particles are generally spherical, noncrystalline, and range from 0.5-5 μm in diameter. [6] Additionally, this technique categorizes the particles on the basis of their elemental compositions. Particles most likely to be associated with GSR are considered "characteristic" and contain lead, barium, and antimony. Particles associated to a lesser degree with GSR are classified as "consistent" with several possible element combinations. [6]

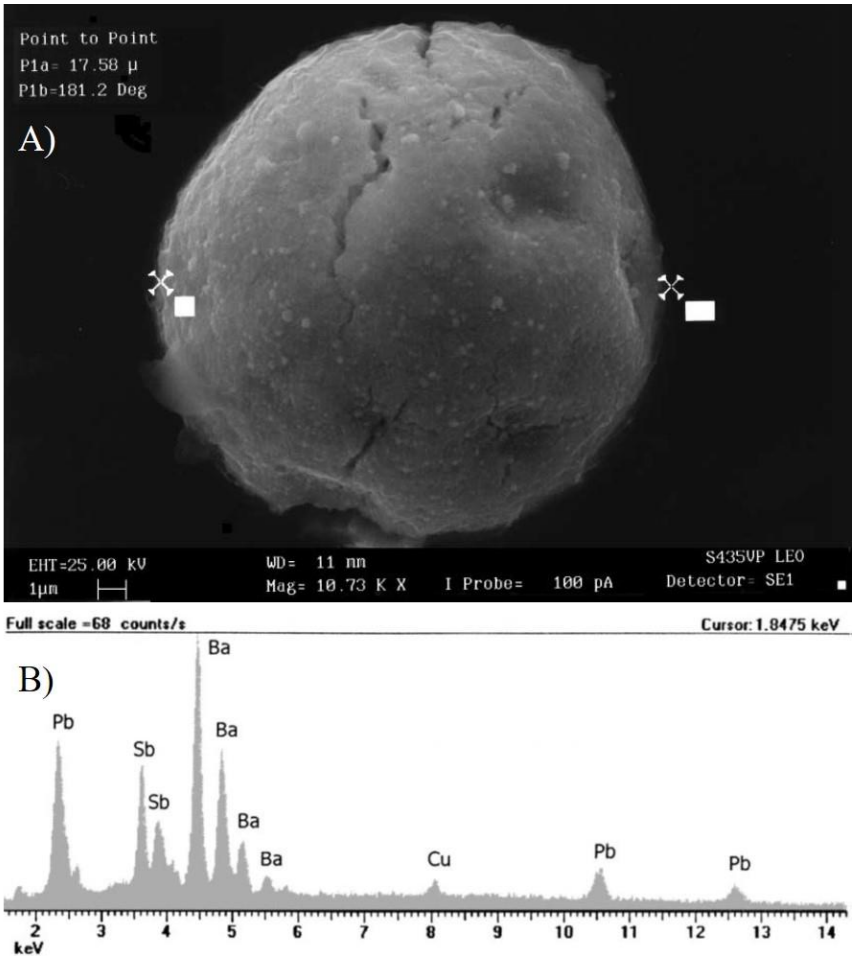


Figure 1. Morphology and elemental composition of GSR particle. A) SEM image of individual particle of GSR. B) EDX spectrum of particle (Y axis is X-ray radiation intensity in pulse counts) showing the elemental composition. [13]

2.3.4. Emergence of Lead-Free Ammunition

The increase in lead-free ammunition worldwide affects forensic scientists who analyze gunshot residue by SEM/EDX since the current identification criteria relies on lead. In light of concerns over its toxicity and negative environmental impacts, manufacturers are gradually eliminating lead in favor of less harmful alternatives. Lawmakers in states such as California are enacting legislation restricting and banning lead-based ammunition for hunting wildlife. [22] As lead-free ammunition becomes more common, forensic scientists must adapt their analysis techniques to reliably confirm the presence of GSR in casework.

The movement to remove lead from ammunition is primarily a result of public health and environmental concerns. Lead poisoning is a direct threat to human health, with temporary and permanent effects throughout the body. Lead can be found predominantly in the bullet itself as well as the primer mixture. Bullets are usually made with lead because of its high density and ease of manufacture. The U.S. National Bureau of Standards estimates that in a firing range, 80% of the lead present comes from the bullet and 20% from the primer. [7] Recreational and professional indoor shooters may develop elevated blood lead levels. [23] Also, lead fragments can remain in hunted big game animal tissues and end up in cuts of meat. [24] Scavenging birds are susceptible to lead poisoning from hunted carcasses, which created the conservation crisis for the California Condor. [22]

Many countries have passed legislation that limits the use of lead-based ammunition. Denmark banned the use of lead shot nationwide for hunting in 1996 and also banned its importation and possession. [25] In 1991, the U.S. Fish and Wildlife Service implemented a nationwide ban on lead shot for waterfowl hunting. California has

spearheaded the movement to reduce lead-based ammunition. Assembly Bill 711 was signed in October 2013 and includes a three-phase implementation gradually mandating non-lead ammunition when hunting wildlife in the state. [17] The U.S. Army is also switching to “green” ammunition. [17]

There are several manufacturers that offer lead-free and non-toxic ammunition in the United States. Barnes Bullets is the largest U.S. maker of lead-free bullets. [26] Almost all of the traditional lead-based bullet calibers used for hunting can be purchased as lead-free. Copper is the typical alternative for bullets and there is no appreciable difference in accuracy or killing power for hunting purposes. Also, competitive pricing will provide an incentive for consumers to switch. [26]

Researchers evaluated lead-free ammunition with current SEM/EDX methods and found several complications. Analysis of Brazilian lead-free ammunition could not provide conclusive identification since the residues produced shared a similar composition with brake dust from automobile components. [7] Additionally, different brands of lead-free ammunition contain varying elemental compositions. In the same study, it was concluded that automated SEM/EDX software may generate false positives and negatives. [27]

Ultimately, the decrease in lead-based ammunition will have an impact on forensic scientists analyzing gunshot residue. Copper-based bullets and lead-free primers will affect SEM/EDX and may provide misleading results. Legislative measures to protect wildlife from lead toxicity will further increase the demand for lead-free substitutes. Gunshot residue analysis methods therefore require updates to reflect the current status of ammunition. A transition towards OGSR analysis is the recommended path forward.

2.4. Organic Gunshot Residue

2.4.1. Components of Organic Gunshot Residue

Organic gunshot residues primarily come from the smokeless powder. The propellant powders are classified by their explosive ingredients. Single-based propellants contain nitrocellulose, double-based also contains nitroglycerin, and triple-based contains nitroguanidine as the third ingredient. [12] Double-based smokeless powders are the most commonly encountered and are used for revolver, pistol, rifle, and shotgun cartridges.

Smokeless powders contain a variety of additives to enhance their properties. Stabilizers are added to prevent self-decomposition and increase the shelf life. Diphenylamine is the most common stabilizer, but centralites may also be used for this purpose. [12] Deterrents include dinitrotoluenes and phthalates which moderate and slow down the powder's burn rate to prevent an explosion. [12] Flash suppressors such as nitroguanidine, nitrotoluene, and dinitrotoluene dilute gases in the muzzle to reduce the brightness. [28] Plasticizers are typically phthalates that reduce hygroscopicity and improve the flexibility of the powder granules. [17]

A recent review article by Goudsmits in 2015 identified a comprehensive list of 136 compounds linked with OGSR, which included various polycyclic aromatic hydrocarbons (PAHs), diphenylamine derivatives, nitrotoluenes, nitrobenzenes, phthalates, and centralites. [5] However, not all of these compounds provide evidentiary value. Polycyclic aromatic hydrocarbons are pollutants found throughout the environment that originate from industrial emissions, vehicle exhaust, and fossil fuel burning. [5] Similarly, phthalates are pervasive in a wide range of household and industrial items. Thus,

from the long list of identified compounds, only those clearly associated with OGSR and not commonly encountered in the environment are suitable for forensic studies.

Unlike IGSR analysis, where important elements are classified by the ASTM E1588 guidelines, OGSR analysis does not have a standard set of compounds for identification. However, Goudsmits recently attempted to classify a set of "characteristic" compounds by their frequency of detection in OGSR combined with a lack of abundance in the general environment. In conjunction with Mach's classification in 1978, Goudsmits identifies nitroglycerin (NG), 2,4-dinitrotoluene (2,4-DNT), diphenylamine (DPA), and ethyl centralite (EC) as "characteristic" of OGSR. [29,30] Individually, these compounds are not limited to OGSR exclusively. Nitroglycerin is a pharmaceutical drug used as a cardiac stimulant, diphenylamine is found in insecticides and perfumes, and 2,4-DNT may be found in azo dyes. [30,31] However, when these compounds are detected together, it is a strong indicator that OGSR is present in the sample. A study of the hands of 100 individuals from the general population did not find these four compounds even with detection limits in the picogram range. [32]

2.4.2. Collection Techniques for OGSR

Numerous collection methods exist for OGSR depending on the collected surface and preferred analysis technique. Tape lifting by dabbing an adhesive on the skin or section of clothing can collect OGSR alongside IGSR simultaneously. However, there are issues with skin debris and fibers that interfere with analysis. [3] Vacuum lifting is primarily for clothing samples with collection of OGSR alongside IGSR using a Teflon filter. Vacuuming shares similar downsides with tape lifting for OGSR since unwanted debris is

also collected. [3] Hair combing is also possible with demonstrated recovery of residues and particles containing NG and EC. [33]

Swabbing is the most extensively utilized collection technique for OGSR on the hands. Typically, a cotton swab or applicator is moistened with a solvent since it improves recoveries when compared to a dry swab. [3] Both organic and aqueous solvents have been employed and studied for this purpose. Organic solvents readily dissolve explosive compounds; ethanol and isopropanol are reported as the top choices. [5] However, they also dissolve skin compounds that cause interferences and therefore require further cleanup. [3] Water was effective and advantageous for OGSR collection with lower detection limits and background noise owing to a cleaner matrix compared to organic solvents. [34,35]

The swabbing region on the hands is also a crucial factor as illustrated in Figure 2. Applicators are brushed on important areas of both sides of the hands such as the palm, thumb, web, and index finger. [36,37] Studies have shown these sampling areas are essential to collect both OGSR and IGSR. Moran and Bell estimate the amount of deposition of OGSR on the hands to vary between 90-178 ng. [38]

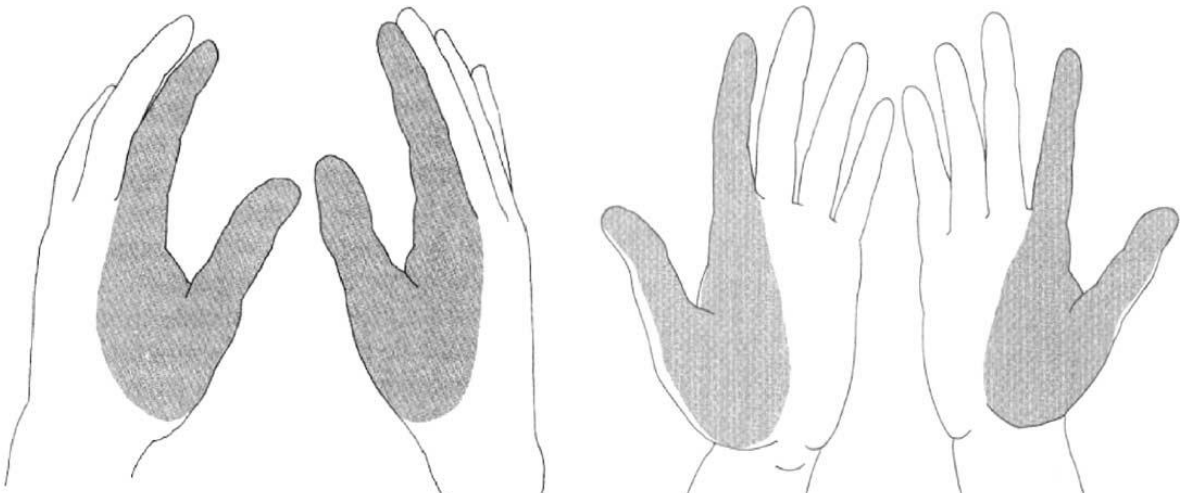


Figure 2. Illustration of important sampling zones for GSR on the hands. [36]

The persistence of OGSR on the hands is another aspect to consider since it depends on numerous factors and influences the amount detected. The amount of GSR deposited from the gun varies depending on the firearm type, ammunition type, amount of shots fired, and wind direction. [12] After initial deposition on the hands, OGSR particles are readily transferred and lost over time; their transient nature greatly complicates collection and analysis. Various actions, such as washing with soap [39], wiping with a towel [12], placing into pockets [40], and handcuffing [41] have been studied and shown to remove OGSR on the hands. A wide range of collection windows demonstrated successful OGSR detection on the hands because of the numerous parameters involved. For example, NG has been recovered on the hands from as few as 0.5 hours [42] and up to 7 hours [43]. Moran and Bell investigated the skin permeation of OGSR on the hands and found they are less prone to secondary transfer compared to IGSR due to their lipophilicity. [38] They also estimated that OGSR can be detected on the hands for up to 24 hours after the firing event.

2.4.3. Extraction Techniques for OGSR

Extraction methods vary greatly since collection techniques for OGSR are so diverse. Sample cleanup and concentration techniques such as solid phase extraction (SPE) are usually required for tape and vacuum lifts since they can collect skin debris and fibers. [3] Without proper cleanup, the sample matrix can contaminate instrumentation and result in high backgrounds and poor limits of detection. Solvent extraction for tape lifts showed low recoveries for OGSR compounds for the commonly employed carbon coated stubs. [44] Organic solvent extraction of hand swabs also dissolved interferences from skin debris. [45] Aqueous solvents avoided interferences but provided lower recoveries and longer extraction times. [5]

Solid-phase microextraction (SPME) offers solvent-free extraction of volatile organic compounds (VOCs) in solid, liquid, or gaseous samples. The SPME technique concentrates volatile analytes of interest onto a fiber coated with a sorbent such as polydimethylsiloxane (PDMS). The procedure avoids unwanted compounds from the sample matrix and bypasses lengthy solvent extraction steps, providing many advantages. Researchers have demonstrated SPME for analysis of smokeless powders and OGSR samples. Joshi used SPME to study the headspace composition of smokeless powders. [46] Dalby optimized the extraction time and fiber type for SPME analysis of unburned smokeless powders. [47]

2.4.4. Analysis Techniques for OGSR

Several instrumental techniques are available for OGSR analysis, although there is no standardized technique as there is for IGSR. Liquid chromatography (LC) with mass

spectrometry (MS) can identify and quantify OGSR compounds. Thomas and McCord used UPLC with tandem MS to identify compositional variations in brands of smokeless powders. [48] Capillary electrophoresis (CE) can separate complex mixtures with high resolution; MacCrehan demonstrated CE for detection of NG in hair samples. [33]

Gas chromatography (GC) in conjunction with several different types of detectors is used extensively for OGSR identification. Gas chromatography is well-suited for volatiles present in OGSR samples. Headspace sampling with SPME fibers followed by thermal desorption is simple and cuts out lengthy solvent extraction steps. [5] Other advantages include rapid analysis time, high sensitivity, low nanogram detection limits, and high specificity. [5] Gas chromatography has been coupled with thermal energy analysis (TEA), [44,45] ion mobility spectrometry (IMS), [49] flame ionization detector (FID), [50] and electron capture detectors (ECD) [51] for highly sensitive OGSR analysis.

2.5. Volatile Organic Compounds (VOCs)

There are several types of compounds in OGSR with various physical and chemical properties. The four "characteristic" compounds mentioned, NG, 2,4-DNT, DPA, and EC, can be classified as volatile organic compounds (VOCs). Volatile compounds tend to evaporate at room temperature. Volatility is measured by a substance's vapor pressure; those with higher vapor pressures evaporate more readily. Substances with vapor pressures above 10^{-2} kPa at 25°C are considered volatile organic compounds (VOCs) and those with vapor pressures between 10^{-2} to 10^{-8} kPa are considered semi-volatile organic compounds (SVOCs). [52] The vapor pressures of the aforementioned OGSR compounds shown in Figure 3 qualify them as SVOCs under these criteria.

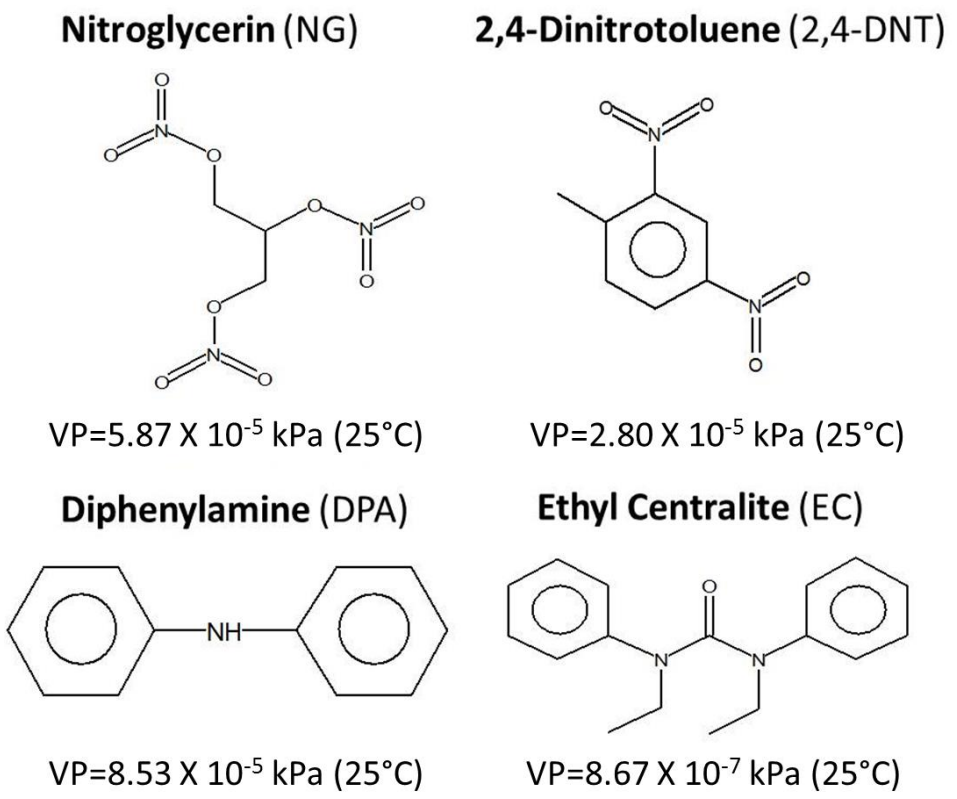


Figure 3. Structures of characteristic SVOCs in OGSR and their vapor pressures. [8]

2.6. Headspace Extraction Technique

Headspace sampling is a relatively simple technique for concentration of volatile analytes. Various applications include blood alcohol determination, wastewater analysis, pharmaceuticals, and perfumes. [53] Volatile analytes of interest are typically injected into a GC with various types of detectors for qualitative and quantitative detection. Sample preparation is minimized since solvent extractions are not typically required. Another key advantage is avoiding unwanted contaminants in dirty sample matrices from entering the GC column.

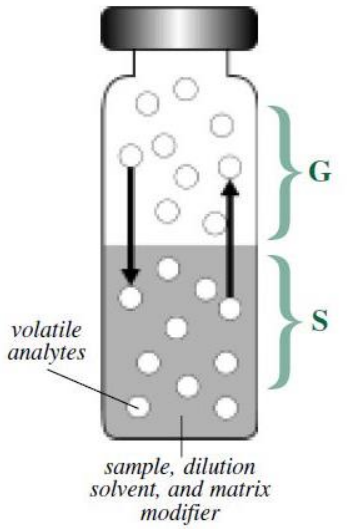


Figure 4. Diagram of headspace vial containing sample (S) and headspace gas (G) showing the equilibrium process between the two phases. [54]

The headspace extraction technique is widely used for its simplicity. Solid or liquid samples are inserted in a sealed container, such as a 5-20 mL glass vial depicted in Figure 4, to prevent escape of volatiles. The sealed vial is kept stationary for a set period of time to allow volatiles to evaporate from the sample and reach a state of equilibrium. Without adequate equilibrium time, the analytes will not be present in the headspace which leads to poor quantitative results. During this equilibrium time, the vial may be heated to increase the analytes' vapor pressure and further increase the concentration in the headspace. [53] The partition coefficient (K) is the ratio of analyte concentrations between the sample (C_s) and gas (C_G) phases. The phase ratio (β) is the ratio of the volumes of the sample (V_s) and gas (V_G) phases. [54] The relationship between these two factors and the concentration in the headspace is also defined in Equation 3 with respect to the original analyte concentration (C_O).

$$K=\frac{C_S}{C_G} \quad (1)$$

$$\beta = \frac{V_G}{V_S} \quad (2)$$

$$C_G = \frac{C_O}{K+\beta} \quad (3)$$

Headspace sampling is performed utilizing either static or dynamic extraction. Static extraction occurs when the air in the headspace is maintained at equilibrium and sampled with a SPME fiber or syringe through the vial septum. [54] In contrast, dynamic extraction occurs when the headspace is transferred to an adsorbent trap by pumping out the air which disturbs the equilibrium. [53] The dynamic purge and trap technique is usually more sensitive because it removes all the air from the headspace. Once extracted, the volatile analytes are transferred to a GC for analysis.

2.7. Gas Chromatography

Gas chromatography separates mixtures of volatile analytes for subsequent detection. Samples are delivered into the injection port as a liquid or gas where analytes are vaporized in the heated inlet. Splitless injection mode delivers the entire sample to the column for trace-level analysis. Split injection mode only delivers a fraction of the sample to prevent detector overload. [55] The analytical column separates the volatile compounds according to their interaction with the stationary phase. The mobile phase is an inert carrier gas, such as helium, that directs the sample through the column. The GC oven containing the column is usually programmed with a temperature ramp to improve the chromatography. [56] Analytes are separated according to their retention time upon exiting the column. Finally, analytes are transferred to one of several different types of detectors for identification, such as a mass spectrometer or electron capture detector.

2.8. Mass Spectrometry

Separated volatile analytes from the GC column are sent through a transfer line to a mass spectrometer (MS) to detect the compounds present. A MS detector operates by ionization of compounds which leads to characteristic fragmentation patterns that identify the original molecule. In electron impact (EI), a hard ionization technique, analyte molecules are bombarded with a stream of 70 eV electrons from the filament in the ion source to fragment them. [57] One common type of mass analyzer is the quadrupole, which filters ions traveling through it by their mass-to-charge (m/z) ratio. [57] Finally, the ions reach the detector to produce a mass spectrum plotting intensity versus m/z ratio. The mass spectrometer is maintained under high vacuum to prevent molecular collisions. Compounds are identified by their unique and distinguishing mass spectra. In full scan mode, the total ion current is obtained, and all of the masses are analyzed. In selected ion monitoring mode (SIM), only specific masses are chosen to provide higher sensitivity. [58]

2.9. Gas Chromatography-Mass Spectrometry for OGSR Analysis

Gas chromatography-mass spectrometry (GC-MS) is regarded as a gold standard in forensic chemistry for its quantitative and qualitative capabilities. In drug analyses, for example, GC-MS is a confirmatory technique for its high specificity that prevents potential false positives from preliminary screenings. Compounds are simultaneously recognized by their retention time and the accompanying mass spectra. A diagram of a typical GC-MS setup is depicted in Figure 5. GC-MS instrumentation is already widespread in forensic laboratories for its applications in toxicology and fire debris analysis, making it an ideal candidate for implementation of an OGSR method. Weyermann analyzed the organic volatile composition of spent 9mm cartridges using SPME and GC-MS to determine the

elapsed time since discharge. [59] Stevens and Bell tested a GC-MS method that required minimal sample preparation since hand swab samples were directly inserted into the inlet for thermal desorption. [60] However, they could not detect NG because of thermal degradation. Overall, GC-MS is an appealing option as an OGSR instrumental analysis technique.

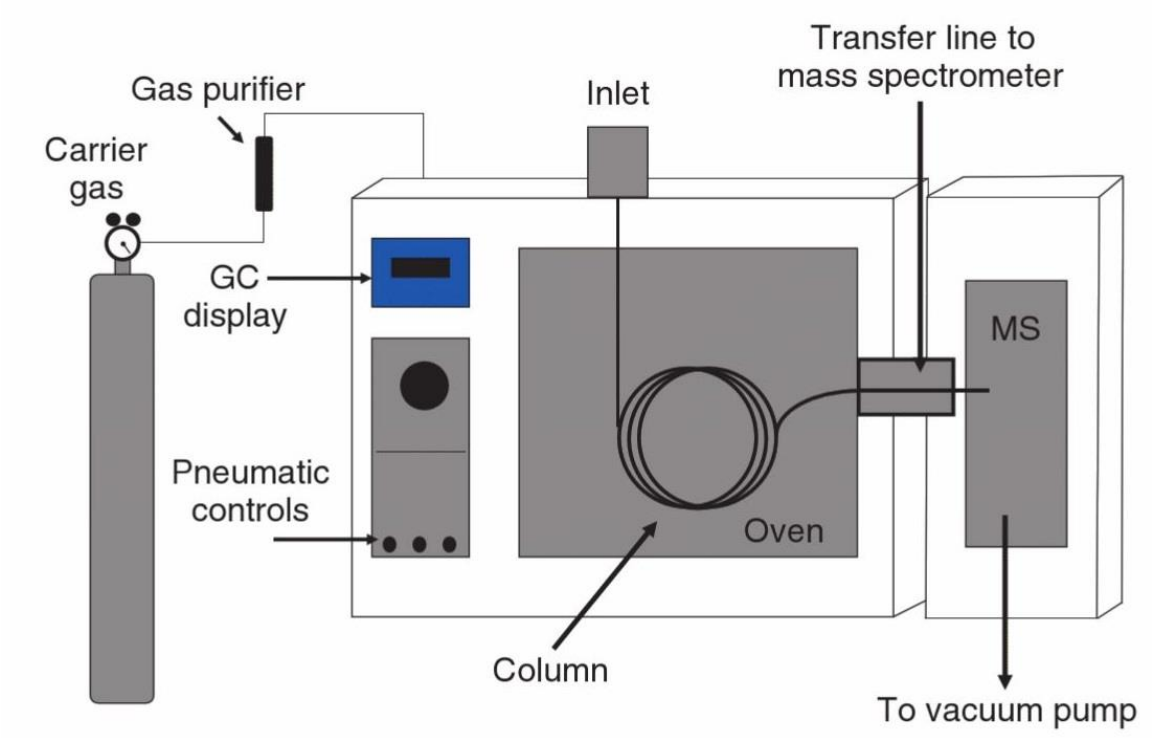


Figure 5. Schematic of a typical GC-MS instrument. [61]

2.10. Capillary Microextraction of Volatiles

The featured technique in this current research project is capillary microextraction of volatiles (CMV), an innovative air sampling device with numerous forensic applications. The CMV was designed and manufactured in Dr. Almirall's research laboratory at FIU's International Forensic Research Institute. The device consists of a glass capillary tube (2

cm X 2 mm) filled with seven rectangular strips (2 cm X 2 mm) of polydimethylsiloxane (PDMS) coated glass fiber filters. The device is open-ended, facilitating the attachment of a vacuum pump to sample air. The manufacturing of the CMV, as well as its precursor, planar solid phase microextraction (PSPME), are described in previous literature. [8,62]

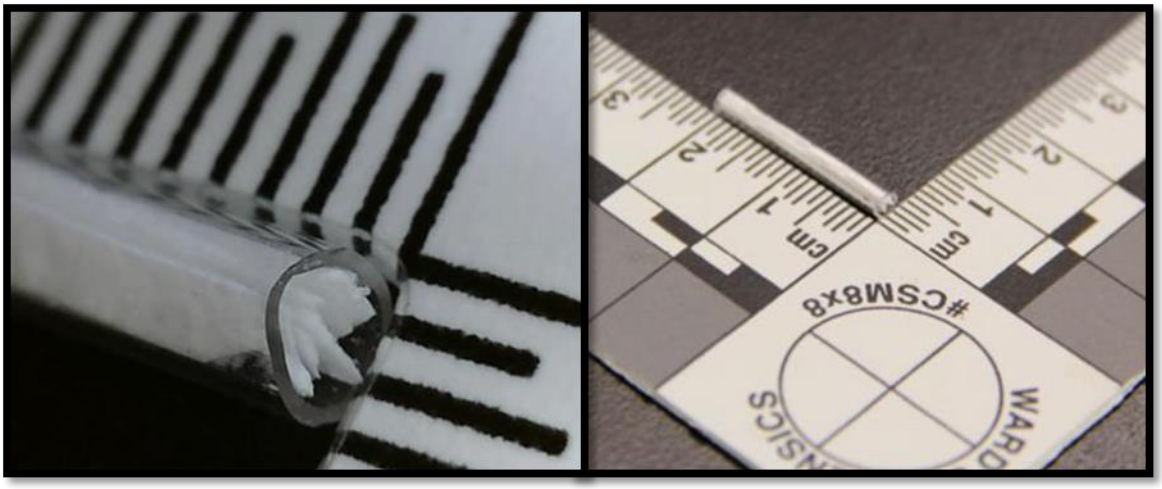


Figure 6. Images of the CMV device showing the dimensions in mm on the left and cm on the right. [8]

The CMV device seamlessly integrates the properties of volatile compounds, the benefits of headspace extraction, and the capabilities of GC-MS instrumentation for qualitative and quantitative analysis. Dynamic headspace extraction is achievable by connecting the CMV to a pump to facilitate extraction of volatiles with little to no sample preparation. Tests showed that even after 60 hours, the CMV can retain over 70% of sampled analytes when sealed in aluminum foil at room temperature. [8] After sampling, the CMV is directly transferred to the GC inlet for thermal desorption of the absorbed volatiles. A commercially available thermal separation probe (TSP) accommodates the CMV for this purpose.

The CMV provides many advantages over traditional sorbent tubes for sampling and analysis of volatiles. Tarifa compared the properties of the CMV and sorbent tubes for extraction of VOCs in indoor air. [63] Common commercially available sorbent tubes are thin steel or glass cylinders packed with a sorbent such as Tenax. Sorbent tubes are limited to low sampling flow rates and may require hour-long extraction times. Also, sorbent tubes require complicated thermal desorption units for introduction to the GC-MS for analysis. The CMV provided faster extraction of VOCs in air within 10 minutes while still providing low nanogram detection limits. [63] Sample introduction of the CMV is a simple process with the thermal separation probe. The CMV is also less expensive (~$1) compared to sorbent tubes (~$100). [10] As a result, the CMV is cost-effective as a disposable sampler or a reusable device with a demonstrated durability of over 100 extractions and injections. [10]

The CMV holds enormous potential for forensic applications involving detection of volatiles. First, Fan showed the CMV's capabilities for detection of VOCs in explosives and smokeless powders. [8] In comparison to a static SPME fiber extraction, the dynamic CMV extraction provided higher sensitivity and shorter extraction times. The improvements are attributed to the CMV's considerably larger surface area (5,000 times greater) and greater phase volume. [8] Additionally, calibration curves generated by direct spike on the CMV and headspace extraction showed linearity and quantitation capabilities. Wiebelhaus identified the volatile components in the headspace above marijuana samples, demonstrating the CMV's potential for illicit drug detection. [64] Tarifa demonstrated the use of the CMV for detecting harmful VOCs in air. [63] Hamblin developed methods to apply the CMV for breath analysis of smokers. [65]

Detection of OGSR on the hands of shooters using CMV-GC-MS was successfully demonstrated by Tarifa. [9] Police officers conducting shooting practice had their hands sampled with cotton swabs moistened with DI water to collect OGSR particles. The swabs were stored in sealed 15 mL glass headspace vials. Static and dynamic headspace extraction of the samples was performed with the CMV device. Both DPA and NG were detected on the hands of 5 out of 9 police officers. [9] The utilized method possessed many desirable qualities, including ease of sample collection, minimal sample preparation, fast extraction time (2 minutes), and low nanogram detection limits. [9] Therefore, the CMV-GC-MS method is a promising technique for OGSR detection on the hands.

2.11. Cryofocusing-CMV-GC-MS

Cryofocusing is an innovation developed for further improvement of the CMV's already excellent extraction capabilities. The concept is similar to cold trapping for focusing of analytes on the head of a column in GC analyses. [56] The CMV is cooled down while air is sampled to increase the extraction efficiency by reducing breakthrough and improving recoveries.

In order to achieve cryofocusing, a custom-built thermoelectric cooler was designed that accommodates the CMV and tubing for the cold extraction. The thermoelectric cooler operates by the Peltier effect, where applying DC current results in heating of one side of a conductive aluminum plate while the other side is cooled. [66] The hot side is connected to a heat sink with a fan, as illustrated in Figure 7. The Peltier device can cool the CMV down to -10°C for the extraction. A thermocouple allows for programmable temperature monitoring and control.

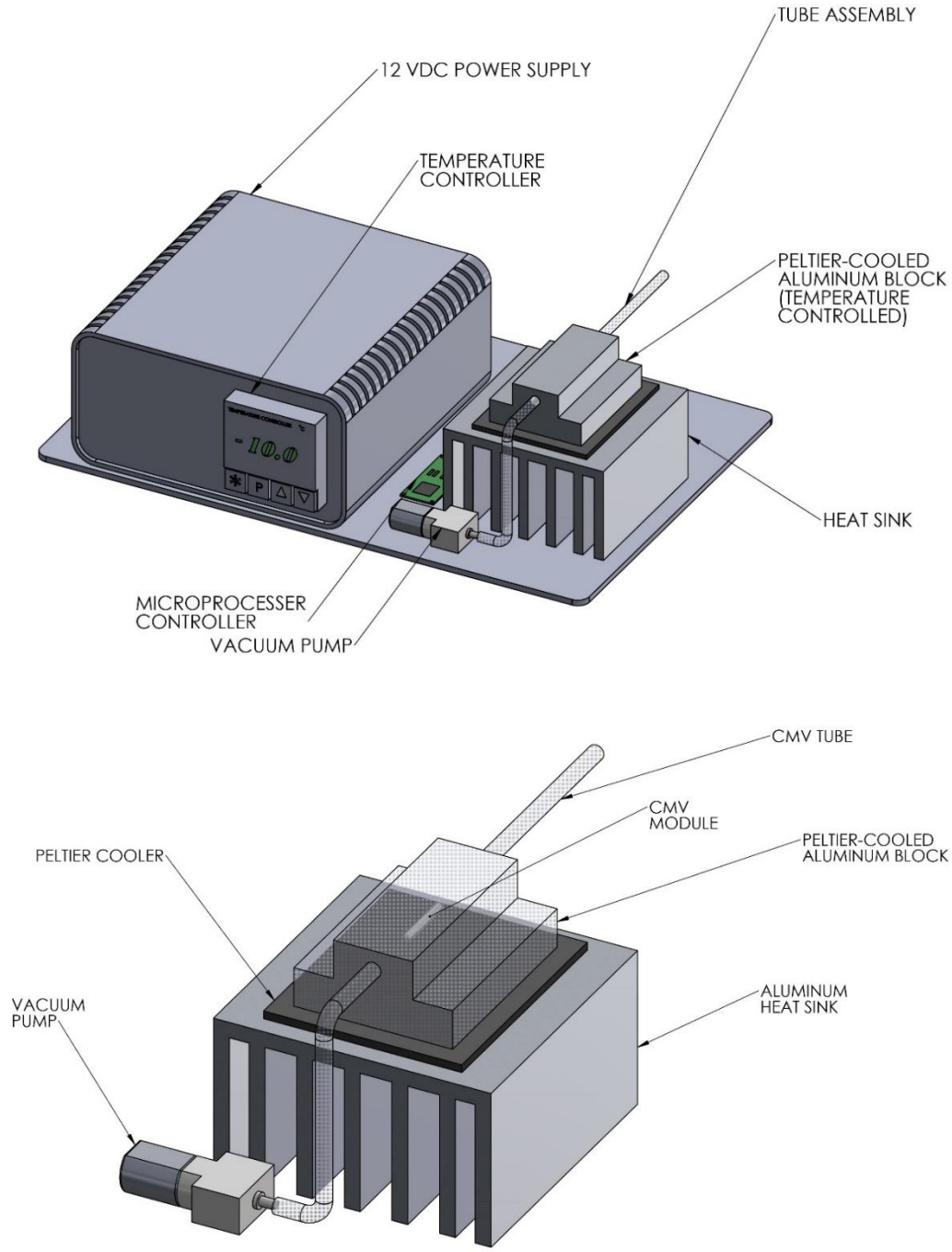


Figure 7. Schematic of Peltier cooler customized for CMV applications with programmable temperature control down to -10°C. Almirall and Tarifa, US Non-Provisional application for "Cryofocused Sampling of Volatiles from Air Using Peltier-Assisted Capillary Microextraction" (US 15/246,866) with the United States Patent and Trademark Office (USPTO).

Cryofocusing capillary microextraction of volatiles (Cryo-CMV) was first evaluated for dynamic headspace extraction of VOCs in air. Comparison of extraction for benzene, toluene, ethylbenzene, and xylenes (BTEX) with the CMV at 20°C versus - 10°C show that cryofocusing improves the amount recovered. [10] Additionally, cryofocusing provided lower breakthrough for most sampled compounds. The results in Figure 8 demonstrate the benefits of cryofocusing for sampling VOCs in air.

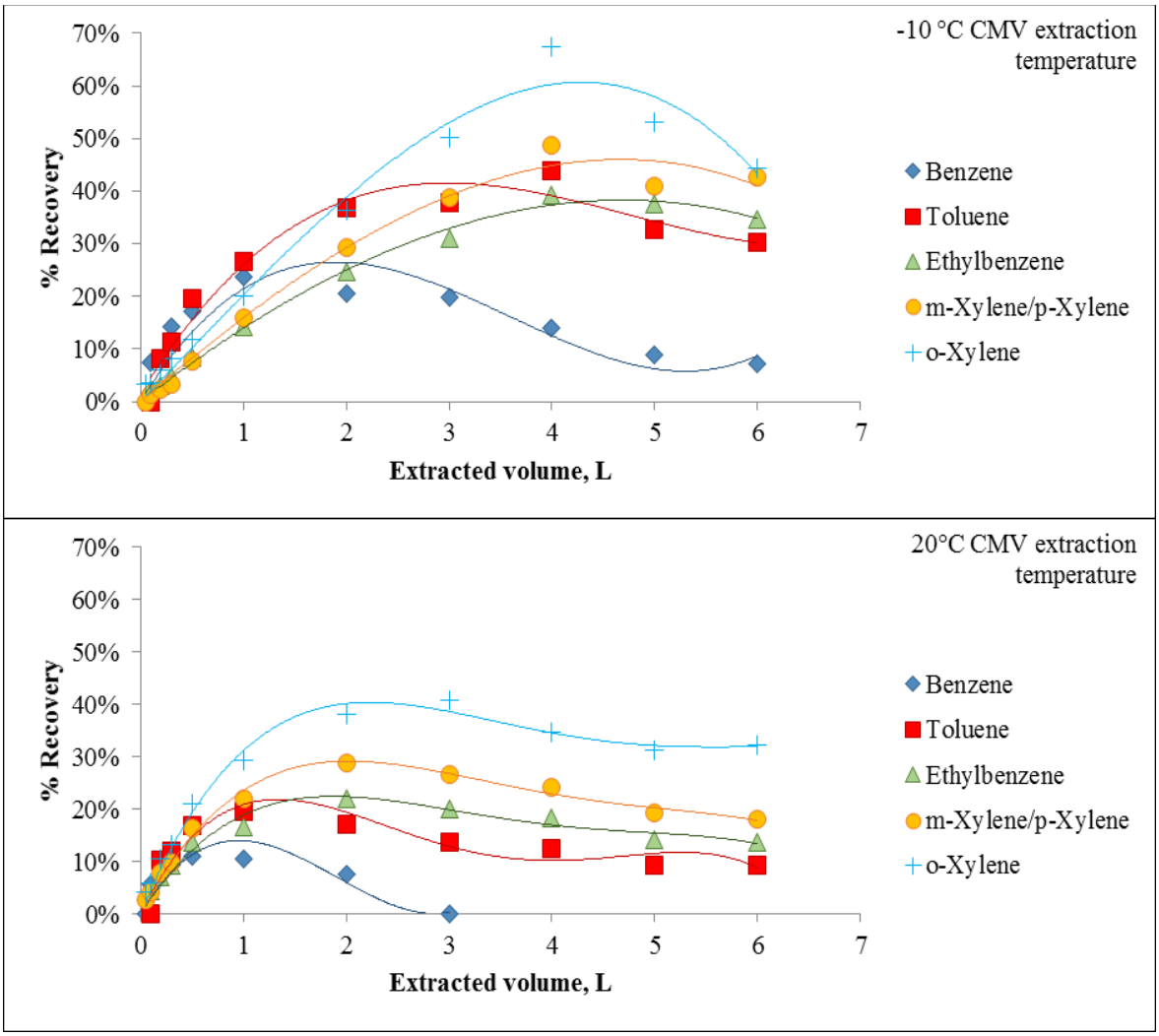


Figure 8. Demonstration of improvement in recovery at different extracted volumes when cryofocusing is applied for extraction of BTEX compounds. [10]

2.12. Research Aims

An increase in lead-free ammunition will complicate IGSR examinations and direct the focus towards OGSR analysis. Currently, there is no universal method for collection, extraction, and analysis of OGSR available. The method developed by Tarifa provides simple collection of OGSR on the hands, rapid extraction with the CMV, and analysis with GC-MS instrumentation that is commonplace in forensic laboratories. [9] The research presented in this thesis aims to further enhance the previously proven CMV-GC-MS technique.

There are three primary motivations and objectives for this research project. First, further method development and optimization experiments for headspace extractions of mock field samples are expected to increase the recoveries of OGSR compounds. Second, incorporation of the Peltier cooler to perform cryofocused extraction of OGSR compounds will be evaluated for the first time. Third, testing the cryofocusing concept for SVOCs in OGSR is important after it demonstrated many benefits for VOCs in air. The knowledge generated will provide further understanding of Cryo-CMV-GC-MS for future application in sampling of ignitable liquid residues, illicit drugs, and exhaled breath analysis.

3. MATERIALS AND METHODS

The organic GSR compounds were purchased as either a standard solution or a solid. Nitroglycerin and 2,4-dinitrotoluene were purchased as 1000 µg/mL standard solutions from Cerillant (Round Rock, TX). Diphenylamine was purchased as a 5000 µg/mL standard solution from Sigma-Aldrich (St. Louis, MO). Ethyl centralite was not available as a standard solution and was purchased as a solid from Sigma-Aldrich (99% purity) and weighed to prepare a stock solution. The DPA and EC were diluted to 1000 µg/mL in HPLC grade methanol. Further dilutions were made from these stock solutions depending on the experiment.

In addition, two different types of smokeless powders were selected for certain experiments. Hodgdon BLC-2 double-based rifle powder contained mostly nitroglycerin and Hodgdon H335 rifle powder contained mostly 2,4-DNT (Hodgdon, Shawnee, KS). The smokeless powders were weighed from their original containers for the experiments.

Two different sizes of containers were employed for storage of the swab samples and smokeless powders. Glass vials (15 mL) with screw caps featuring either red rubber/PFTE or silicone/PFTE septa for an airtight seal were purchased from Supelco (Bellefonte, PA). Metal cans (~1 L) with accompanying airtight lids were purchased from All-American Containers (Miami, FL). The cans were baked out in an oven at 250°C for three days prior to use in order to remove any potential background contaminants. The lids were punctured to create uniform round holes and sealed with rubber sleeve septa from Ace Glass (Vineland, NJ) to accommodate headspace sampling.

Different types of air sampling pumps were utilized for the headspace extractions. A Bailey Nurture III pump was capable of extracting at a flow rate of 0.2 L/min. An Escort ELF (Zefon, Ocala FL) portable air sampling pump with digital readout was capable of extracting at flow rates between 0.5-3 L/min. These pumps were connected to the CMV and container by either Tygon or Teflon tubing with the appropriate dimensions to ensure an airtight connection. Additionally, a custom handheld pump from Air Chemistry specifically designed to accommodate the CMV with a flow rate of ~0.7 L/min was tested for its direct sampling capabilities.

3.1. Capillary Microextraction of Volatiles (CMV) Device

The capillary microextraction of volatiles (CMV) devices were made and assembled in the laboratory. A set of three CMVs was manufactured according to the protocol described previously. [8] The CMVs were weighed after assembly (0.262g ± 0.00351g) to check their uniformity. The CMVs were preconditioned in an oven at 250°C overnight prior to first use; thereafter, they were preconditioned for 30 minutes at the same temperature prior to each experiment and desorbed as a blank run in the instrument. The CMVs were stored in aluminum foil when not in use. An image of the CMV device is presented in Figure 9.

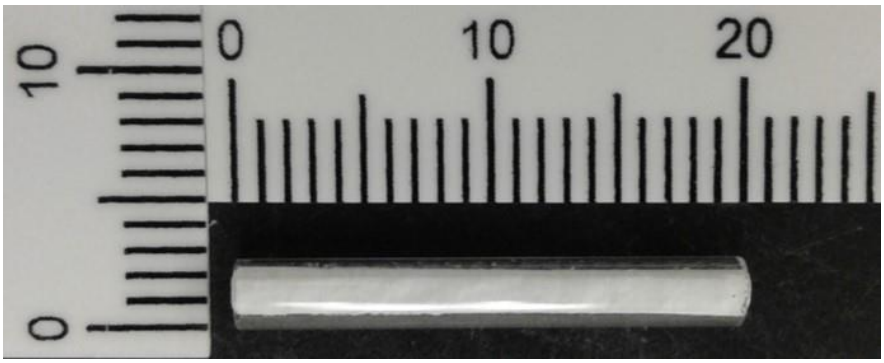


Figure 9. Picture of the CMV device which measures 2 cm long with a 2 mm diameter.

3.2. Peltier Cooler for Cryofocusing

A thermoelectric cooler was built for cryofocusing of the CMV during the headspace extraction. The device was custom-built and designed to fit the dimensions of the CMV. The parts included a 12 V power supply, heat sink with fan (Adafruit, New York, NY), Omega CNi16D temperature controller with type K thermocouple probe (Omega, Samford, CT), and a 4 cm X 4 cm Peltier cooler module. An aluminum block was drilled to make a hole designed to fit the CMV inside with the tubing going to the pump. The hot side of the Peltier cooler module was attached to the heat sink and the cold side was attached to the aluminum block. The cooler module was covered with thermal paste on both sides, which was found to be critical for ensuring good conductivity and achieving a stable desired temperature. The controller can be programmed to the desired setting, down to the lowest achievable temperature of -12°C.

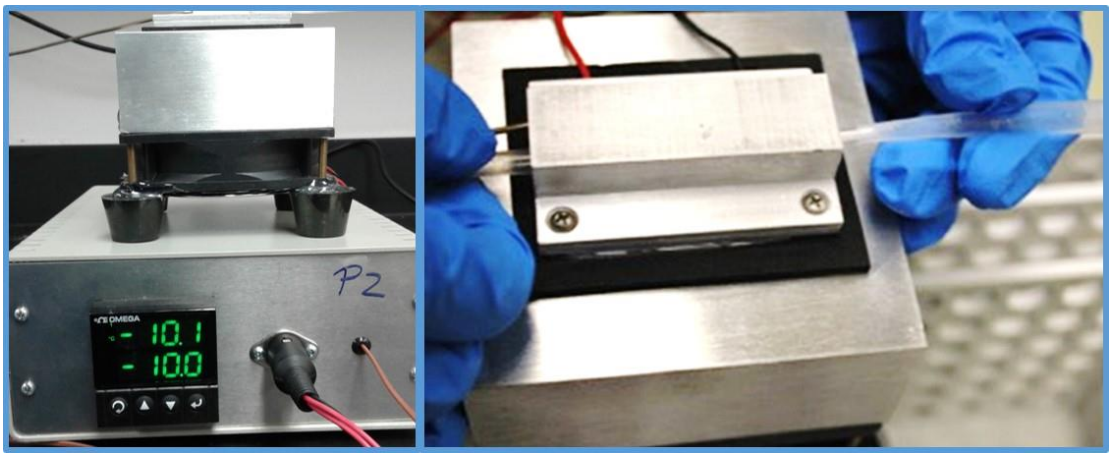


Figure 10. Peltier cooler module with temperature control device. The CMV is guided through the hole on the aluminum block as shown. USPTO Non-provisional application (US 15/246,866) [67]

An experiment was conducted to determine the time required for the Peltier device to cool down the CMV to the desired temperature. Adequate cooling time is important for ensuring the CMV is at the correct temperature during the extraction. First, the cooler was

turned on, with the tubing inside (but not the CMV), for five minutes to cool it down to -11°C while measuring with a thermocouple probe. Next, the CMV was placed inside the tubing in the aluminum block and the temperature was recorded with a second thermocouple probe placed in the CMV. The results in Figure 11 show that approximately two minutes are required for the CMV to fully cool down. The temperature is maintained for at least five minutes, which is more than enough time to perform the headspace extraction.

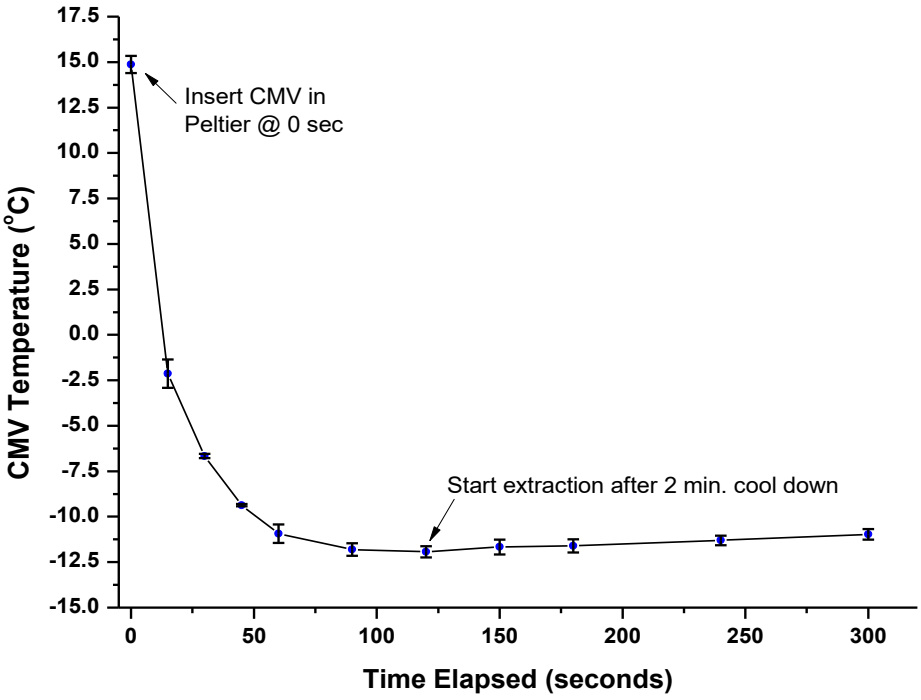


Figure 11. Temperature curve of the cooling process of the CMV when inserted into a pre-cooled Peltier cooler starting at -11°C; 3 replicate measurements were taken and indicate a reproducible cooling mechanism for the experiments.

3.3. Headspace Extraction Setup

There were two different headspace extraction setups in the laboratory, which depended on whether the vials or the cans were analyzed. In certain experiments, heating of the sample container was necessary to increase the concentration in the headspace. Vial

samples were placed inside a water bath heated on a hot plate as depicted in Figure 12. The can samples were placed inside a Glas-Col PL 100D can heater with a 104A PL612K Digitrol II digital temperature control with thermocouple input (Glas-Col, Terre Haute, IN). Either the Bailey or Escort ELF sampling pumps were utilized for the extraction and set to the desired flow rate. The pumps were connected via Tygon or Teflon tubing of the appropriate dimensions. The setup was designed to accommodate the Peltier cooler device. The CMV was placed in the tubing and directed through the slot in the Peltier cooler metal block. A disposable stainless steel 16-gauge needle was connected to the end of the tubing to puncture through the septa and extract the headspace. A second 16-gauge needle was also inserted to allow for air flow into the vial and prevent a vacuum buildup. The CMVs were analyzed immediately after the extractions. A blank of the entire setup was run prior to the experiments to ensure there were no interferences. The tubing and needles were cleaned in between extractions with methanol and flushed with high purity air or nitrogen.

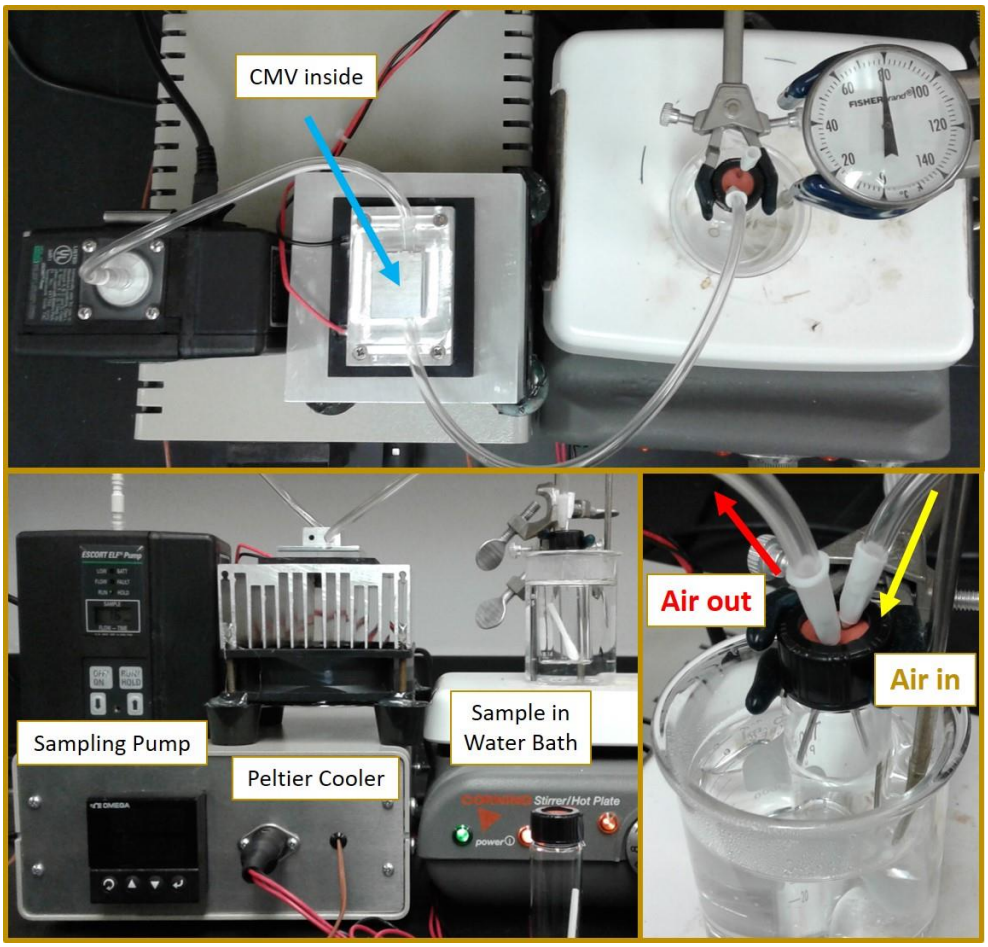


Figure 12. Different views of the headspace extraction setup for vials. Includes Escort ELF pump, water bath to heat vials, and Peltier cooler for cryofocusing of CMV.

3.4. Instrumentation

Analysis was performed using an Agilent 7890A gas chromatograph connected to a 5975C Inert XL single quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA). The instrument also features a micro-electron capture detector (μECD). The parameters followed previous research by Fan and Tarifa for OGSR analysis on the same instrument. [8,9] The acquisition method was set to collect full scan, selected ion monitoring (SIM), and ECD signals. A split/splitless inlet was installed for thermal desorption with a Restek 4mm ID single taper liner. The column installed was a DB5-MS Ultra Inert (5.8m X 0.25mm X 0.25μm). The shorter length column was found to perform

better for explosives and GSR compounds. Ultra-high purity helium was used as the carrier gas at a flow of 1.2 mL/min through the column. The details of the GC method are also listed in Table 1. The instrument was tuned as recommended by the manufacturer prior to the experiments using the autotune feature. Instrument blanks, CMV blanks, and control solutions were analyzed prior to each experiment.

Table 1: General parameters for the GC acquisition method used for the experiments.

Inlet Temp: 180°C	Flow: 1.2mL/min	Split (5:1)
Transfer Line: 280°C	Ion Source: 230°C	Quadrupole: 150°C
Temp. Program (21.17 min total)	**Temp**	**Hold**
Ramp	Initial: 40°C	0.5 min
15 °C/min	240°C	5 min
30 °C/min	280°C	1 min

3.4.1. Thermal Separation Probe

In order to facilitate thermal desorption of the CMV directly in the injection port of the GC, a commercially available Agilent Thermal Separation Probe (TSP) was mounted in the front inlet. A Restek 4mm ID single taper liner was installed in the injection port. The adapter is typically utilized for insertion of microvials into the inlet for analysis of solid or slurry samples with complex matrices. The CMV is placed in the slot of the probe and the assembly is inserted into the inlet for thermal desorption as portrayed in Figure 13.

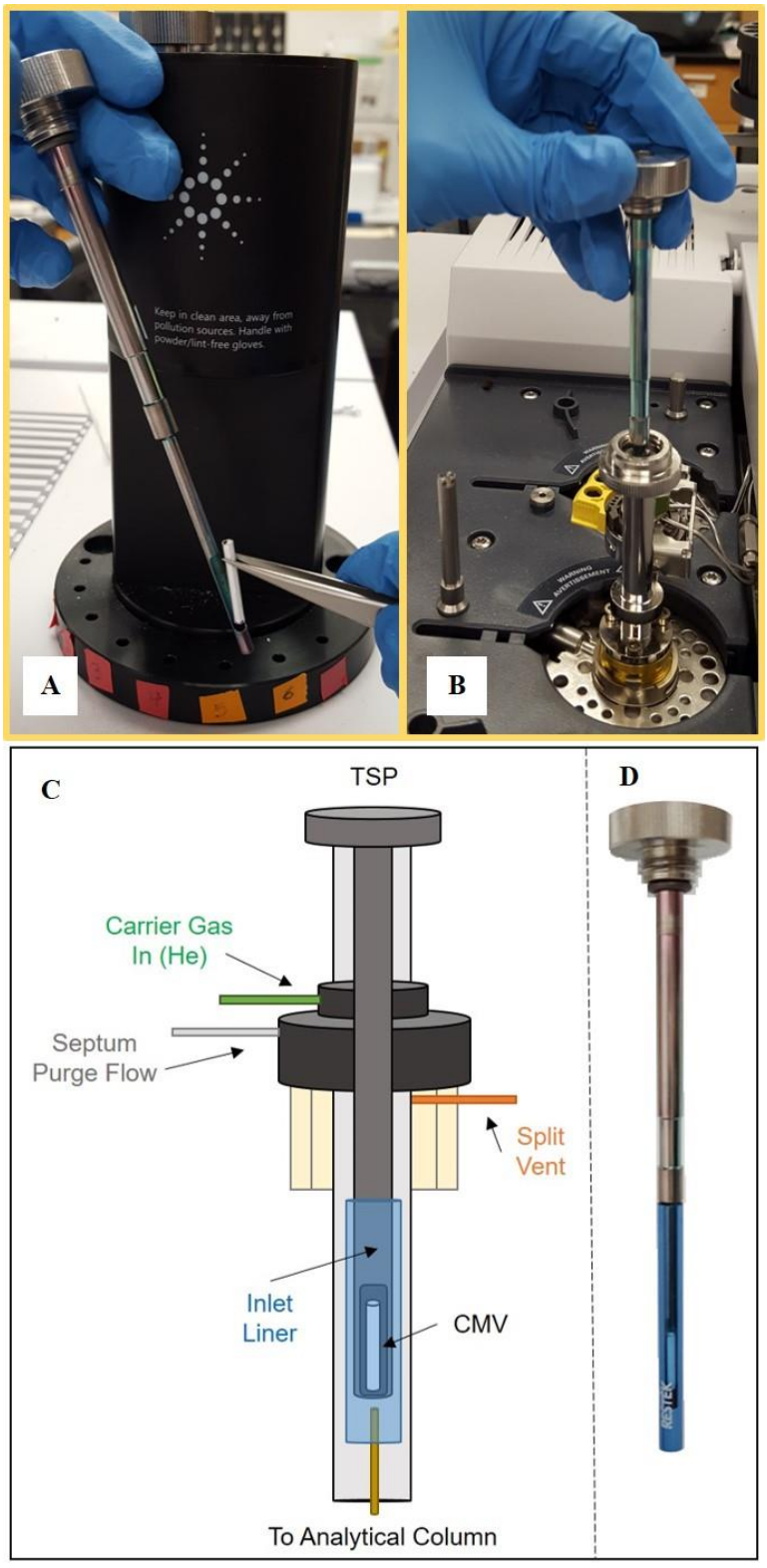


Figure 13. A) Insertion of CMV into TSP. B) Insertion of TSP into GC inlet. C) Diagram of TSP with relevant parts. D) Picture of the actual TSP showing the liner and CMV inside.

3.4.2. Data Processing and Analysis

The GC-MS method included the collection of total ion current (TIC), selected ion monitoring (SIM), and electron capture detector (ECD) data simultaneously. An initial test run of the four compounds of interest revealed the retention times. The SIM ions and windows are described in Table 2. The SIM mode was utilized frequently due to the larger background in the TIC that could obscure peaks with lower signal intensities. Extracted ion chromatograms were obtained from the full scan mode with the same qualifier ions listed below. Data analysis was conducted using the accompanying Agilent ChemStation v. E.02.01.1177 software, Microsoft Excel, and Origin Pro 8. The ChemStation software obtained and processed chromatograms, integrated peak areas, and signal-to-noise ratios. Manual peak integration was performed in a consistent manner to obtain accurate peak areas for quantitation.

Table 2. Details of the GC-MS method for operating the MSD in SIM mode.

Compound	Retention Time (min)	SIM Window (min)	SIM Ions (m/z)
NG	6.076	4.000-7.000	46, 76
2,4-DNT	7.413	7.000-7.900	63, 89, 119, 165
DPA	8.117	8.000-9.700	167, 168, 169, 170
EC	9.901	9.700-11.000	77, 120, 148, 268

3.5. Field Sampling Protocol

Institutional Review Board approval (IRB-16-0277) from Florida International University was granted for collection of field samples for the current study. Hand samples were obtained using cotton swabs moistened with 18 MΩ DI water to avoid potentially harmful organic solvents. The back of the index finger, thumb, and palm areas are important to swab, as described in previous literature. [36] Police officers conducting shooting practices at a training facility (Miami-Dade Public Safety Training Institute,

Doral, FL) were asked for verbal consent prior to collection of samples. Hand blanks were collected prior to handling of the firearm. Volunteers at FIU who had not previously touched a firearm were also asked to swab their hands in a similar manner. All samples were immediately stored after collection in either the 15 mL vials or 1 L cans at room temperature and returned to the lab for analysis.

4. EXPERIMENTAL RESULTS AND DISCUSSION

4.1. Inlet Temperature Optimization

The temperature of the inlet for thermal desorption of the CMV is a crucial parameter that requires optimization. The inlet temperature must be high enough to ensure desorption of the compounds off the CMV, but low enough to prevent thermal degradation. A range of different inlet temperatures (160-200°C) was tested to find the most suitable one for the OGSR compounds. A 15 ppm mixture of NG, 2,4-DNT, DPA, and EC was prepared in methanol. A direct spike onto the CMV of 1 µL of the mixture (15 ng loaded) was performed and the CMV was immediately placed in the inlet using the TSP to start the run.

The results presented in Figure 14 show that increasing the inlet temperature within this range improves the sensitivity for certain compounds. The three compounds that showed an improvement with increased temperature, 2,4-DNT, DPA, and EC, have high boiling points (250, 302, 330°C, respectively) that are above the inlet temperature setting. Nitroglycerin, however, is widely reported as a thermolabile compound. [68] It is susceptible to degradation in the inlet at high temperatures, which was observed here above 180°C. Weyermann also reported degradation of NG above 180 °C in the GC inlet. [59] The peak height and areas for NG are the lowest of the four compounds so improving the sensitivity for NG is crucial. Although higher temperatures provided better sensitivity for the other three compounds, it was more important to improve the sensitivity for NG, so the inlet temperature was set to 180°C for future experiments.

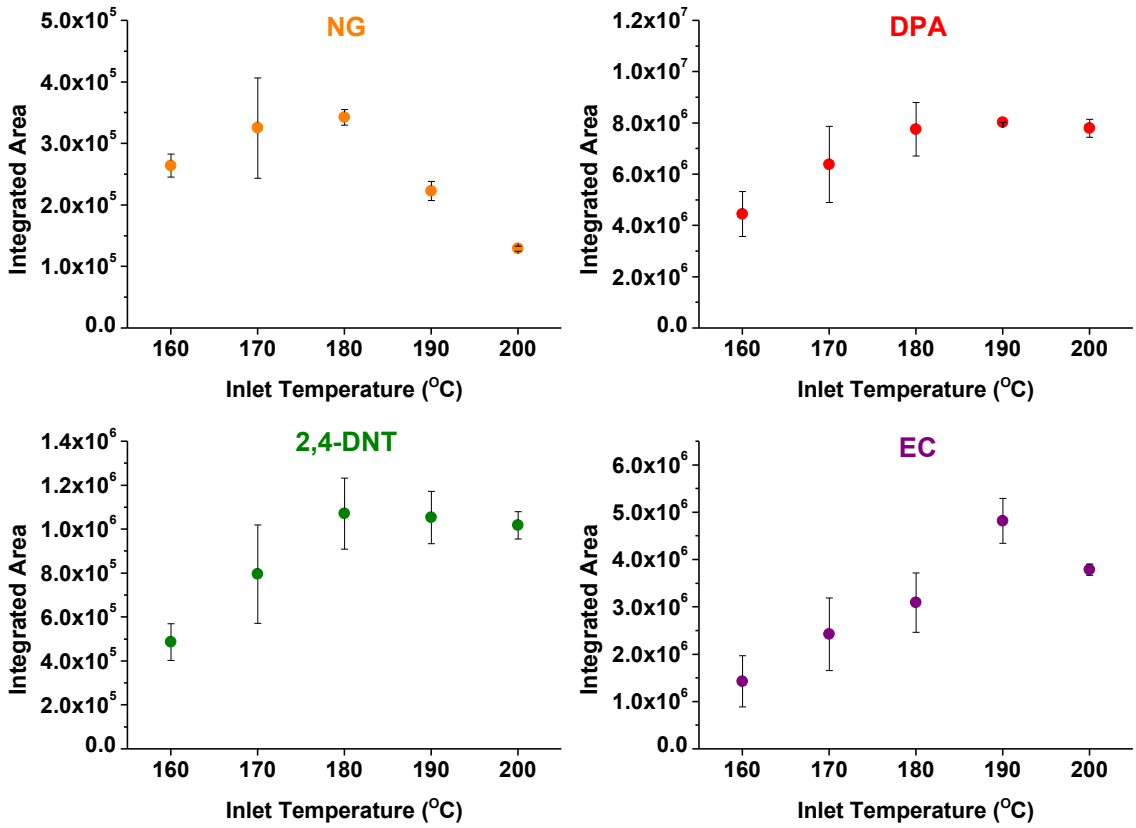


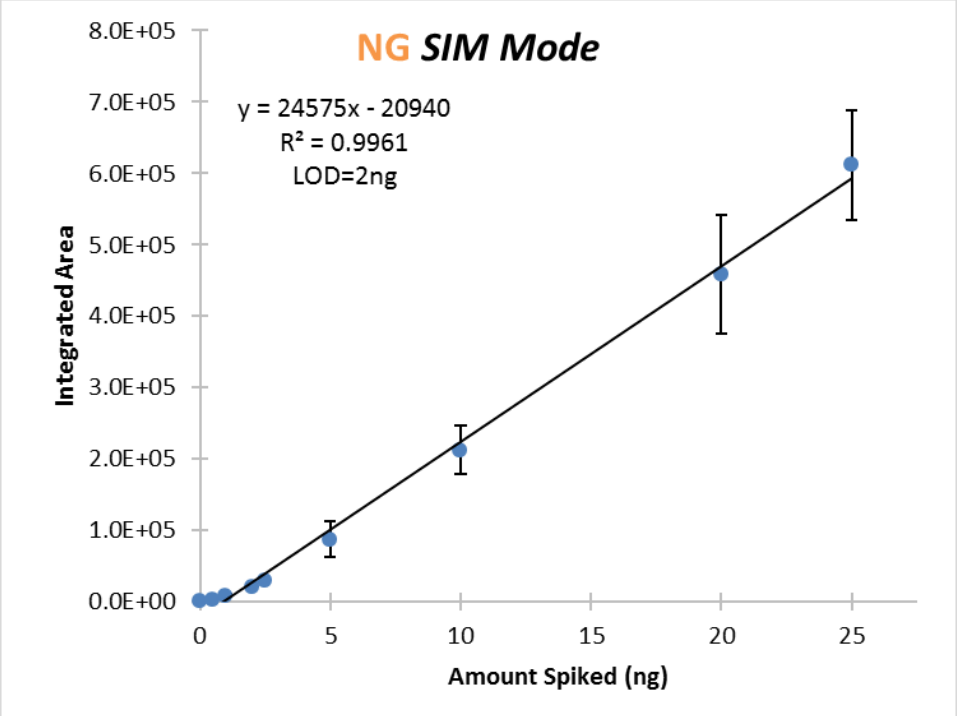
Figure 14. Inlet temperature optimization for OGSR compounds using integrated peak areas in SIM mode. 15 ng direct spike on CMV (n=3 replicates)

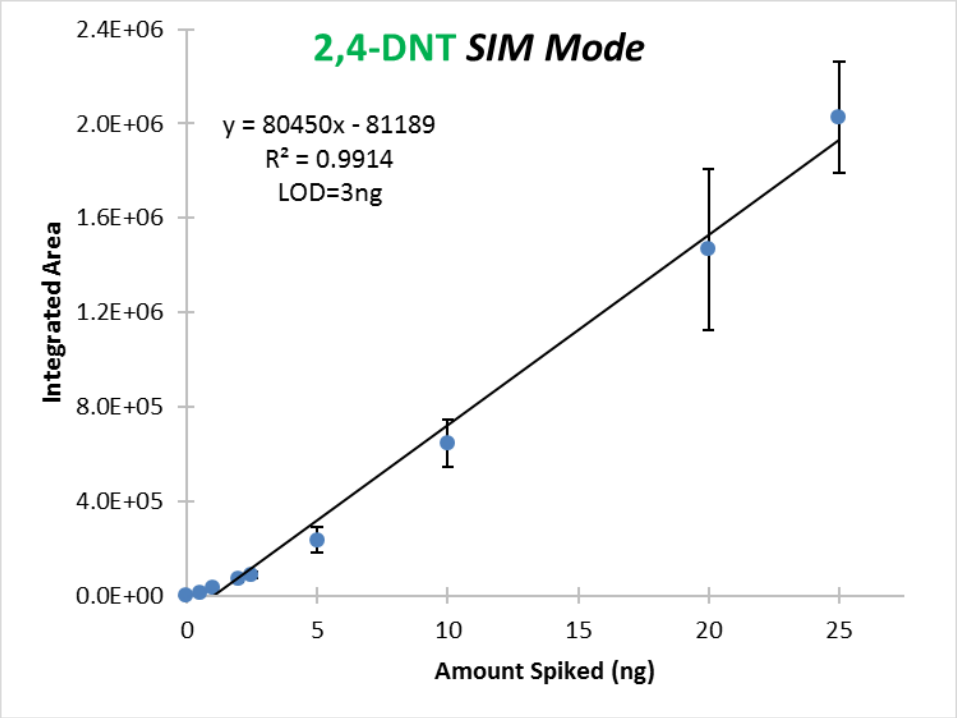
4.2. Direct Injection Calibration Curves

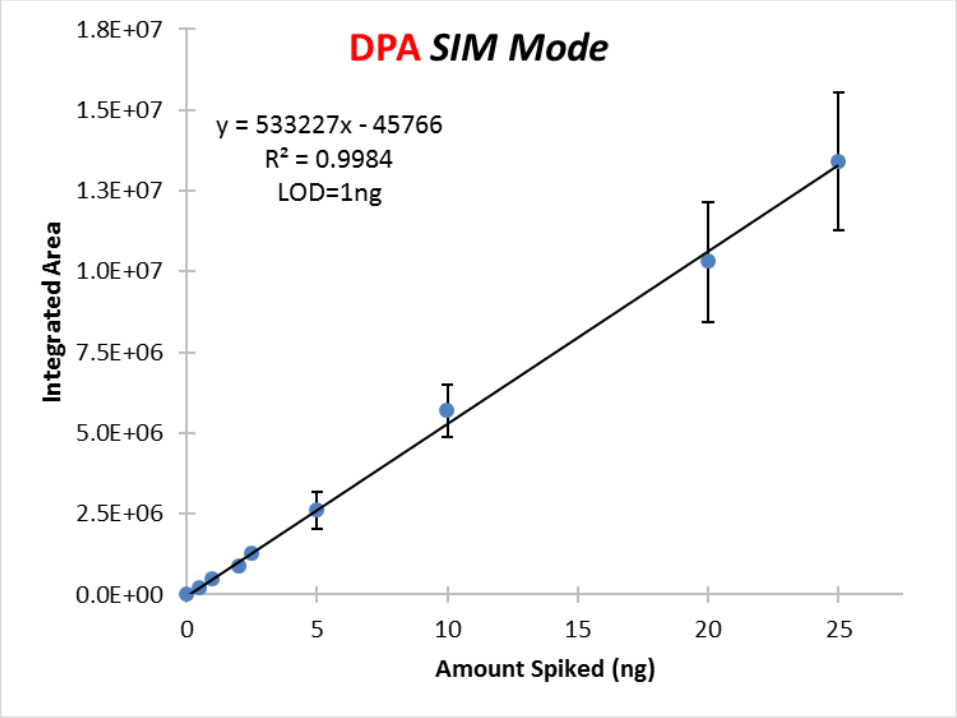
A calibration curve derived from spiking solutions directly on the CMV was created to facilitate the calculation of recoveries from the headspace extractions. Stock solutions of NG, 2,4-DNT, DPA, and EC were prepared at 1000 µg/mL in HPLC grade methanol. A range of calibration solutions with a mixture of these four compounds in equal ratios were prepared by diluting to 0.5-25 µg/mL in methanol. A direct spike of 1 µL of the solution was deposited on the PSPME strips of the CMV (avoiding the glass) and immediately placed in the GC inlet via the TSP to start the run. A blank spike on the CMV using only methanol served as the 0 ppm point. The integrated peak areas were obtained and plotted

versus the amount spiked to generate a calibration curve. The regression equations, limits of detection (LOD), and R^2 values were determined as well. The LODs were calculated using the standard error of y ($S_{y/x}$) function in Excel and the slope (m) with the equation below.

$$LOD = 3 \times \frac{S_{y/x}}{m} \quad (4)$$




2,4-DNT SIM Mode
y = 80450x - 81189
R² = 0.9914
LOD=3ng
Integrated Area
2.4E+06
2.0E+06
1.6E+06
1.2E+06
8.0E+05
4.0E+05
0.0E+00
0
5
10
15
20
25
Amount Spiked (ng)


DPA SIM Mode
y = 533227x - 45766
R² = 0.9984
LOD=1ng
Integrated Area
1.8E+07
1.5E+07
1.3E+07
1.0E+07
7.5E+06
5.0E+06
2.5E+06
0.0E+00
0
5
10
15
20
25
Amount Spiked (ng)

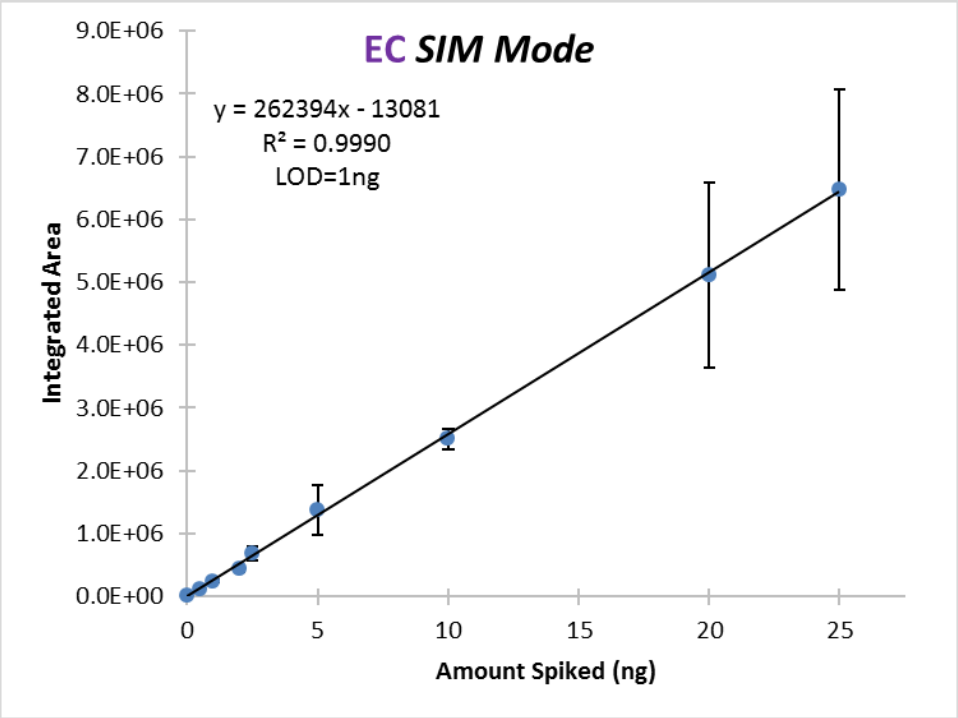


Figure 15. GC-MS calibration curves in SIM mode for direct spike on CMV for NG, 2,4-DNT, DPA, and EC (n=3 replicates)

The calibration curves presented in Figure 15 indicate good linearity (>0.991) and provided the ability to quantitate the amount recovered when performing headspace extractions with the CMV device.

4.3. Initial Testing of Headspace Extraction Parameters

In order to facilitate the Peltier cooler, the headspace extraction setup required modifications and optimizations from the previous parameters. These were implemented from prior work by Tarifa on the same compounds, which consisted of heating 15 mL vials for 20 minutes in a semi-closed system at 65°C followed by extraction at 1 L/min for 2 minutes. [9] Initial experiments were performed to optimize these parameters and improve the recoveries for the extractions in the vials and cans.

The optimization experiments were conducted with changes to mimic the field samples. Previously, experiments were performed by spiking solutions directly on the bottom of the vial. Instead, to simulate actual samples, cotton swabs were moistened with a consistent amount of DI water (i.e. 30 µL) and placed inside the vials. A known amount of a mixture of OGSR compounds diluted in methanol was spiked directly on the wet swab. Moran and Bell estimated that between 90-178 ng of OGSR compounds are deposited on the hands of shooters. [60] On the basis of their calculations, an appropriate amount was spiked on the cotton swabs depending on the experiment.

Preliminary experiments were conducted by spiking a mixture of 100 ng each of NG, 2,4-DNT, DPA, and EC on a moistened swab in the 15 mL vials. However, when the vials were kept at room temperature, there was difficulty in detecting the compounds in the headspace when 100 ng was spiked on the swabs. Therefore, heating the vials was necessary to prevent trapping of the compounds on the cotton, encourage their evaporation, and promote a higher concentration in the headspace.

4.4. Experiments with Vial Samples

4.4.1. Heating Temperature Optimization in Vials

The setup was altered to include a water bath for improved temperature control and homogenous heating of the entire vial. An experiment was performed with a 100 ng spike (10 ppm mix X 10 µL) each of NG, 2,4-DNT, DPA, and EC deposited on a cotton swab moistened with DI water and inserted in the 15mL headspace vials. The heating time was fixed at the optimum 20 minutes and the CMV was maintained at room temperature for extraction at 1 L/min for 2 minutes. The results in Figure 16 indicate that heating to 80°C

achieves the highest sensitivity. Higher temperatures above 85°C were not recommended due to moisture buildup on the tubing that affected the extractions.

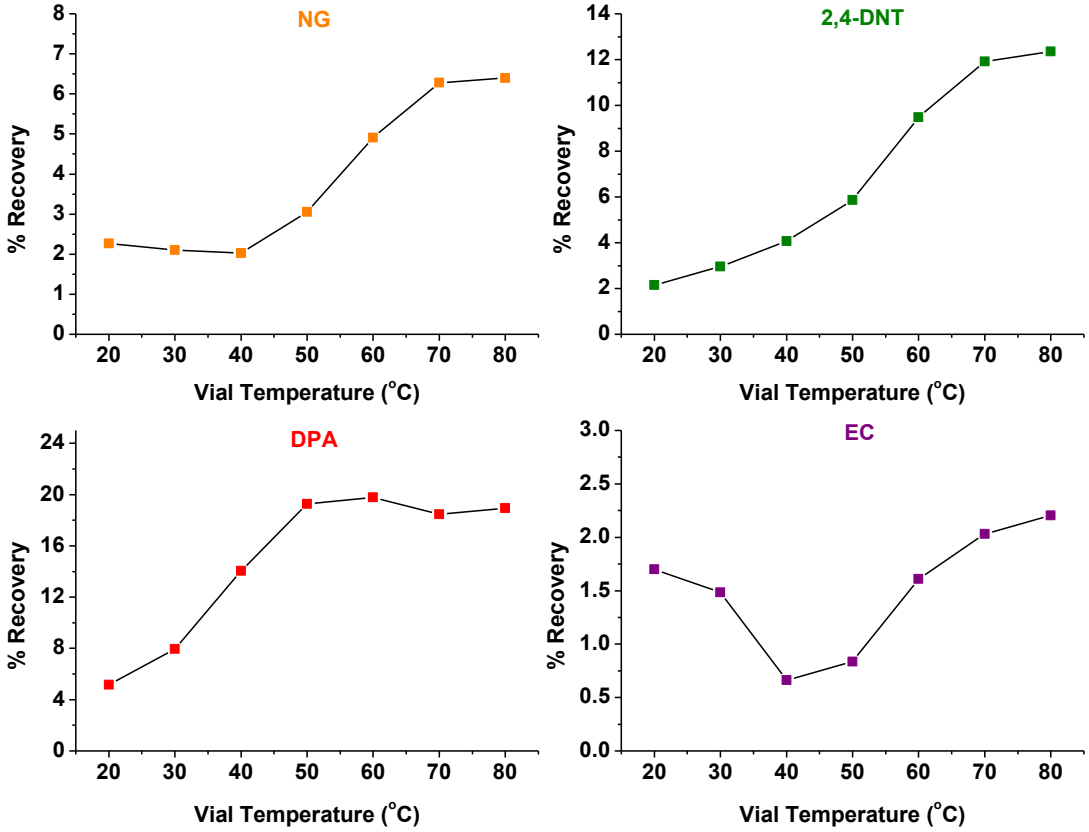


Figure 16. Heating temperature optimization for extraction of 15 mL vials. Parameters: 100 ng spike of each compound on cotton swab moistened with DI water. Equilibrium time: 20 minutes at different temperatures. Flow rate: 1 L/min. Sampling time: 2 min. CMV temperature: 20°C.

4.4.2. Sampling Volume Optimization in Vials

The extracted volume from the vial also required optimization. An adequate extraction time is required to ensure as much of the headspace is sampled without incurring breakthrough. For this experiment, 100 ng was spiked on a moistened cotton swab and heated to the previously optimized 80°C for 20 minutes prior to extraction. The sampled volume is a function of the pump flow rate and the extraction time. For example, to sample 3 L of headspace, the flow was set to 1 L/min and the vial was extracted for 3 minutes.

$$Sampled\ Volume\ (L) = Flow\ rate\ \left(\frac{L}{minute}\right) \times Sampling\ Time\ (minutes) \qquad (5)$$

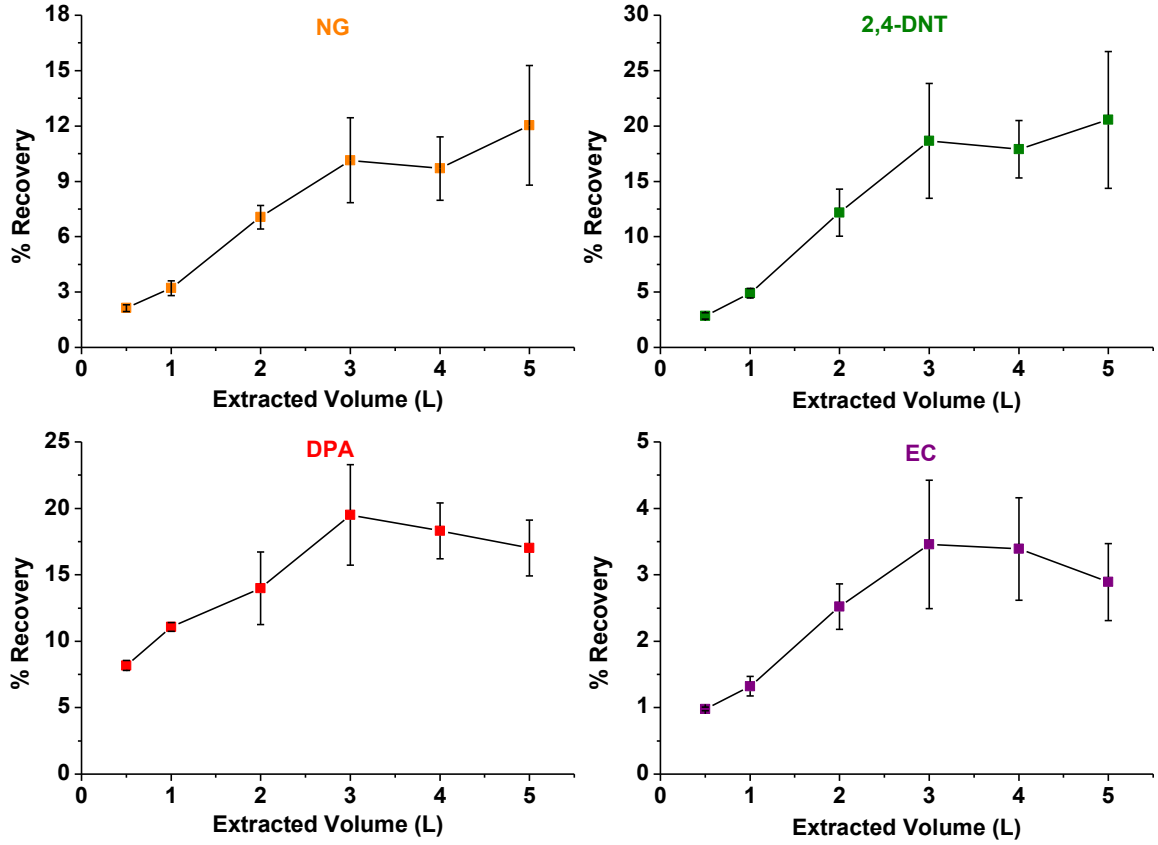


Figure 17. Extracted volume optimization for 15 mL vial samples. Parameters: 100 ng of each compound spiked on DI water moistened cotton swabs. Heating time: 20 minutes. Heating temperature: 80°C. Flow rate: 1 L/min. CMV temp.: 20°C. (n=3 replicates)

Several conclusions were reached from this experiment. First, it was observed that despite having a vial with only 15 mL of headspace, the recovery of the compounds increases up to 3 L of extracted volume. Also, after extracting 3 L, there is a plateau and decrease in the recovery for DPA and EC; this is most likely due to breakthrough of the compounds. The optimal sampling volume for highest recoveries was 3 L, although 2 L provides better overall precision for most compounds. Therefore, either 2 L or 3 L sampling of the vial was considered for future experiments.

4.4.3. Comparison of Old to New Setup

The initial parameters were compared to the optimized parameters to illustrate the improvements in recovery for the new setup. The tubing material of the transfer line connecting the vial to the CMV significantly affected the extractions; changing from Tygon to Teflon tubing greatly increased the recoveries. The improvement is seen in Figure 18.

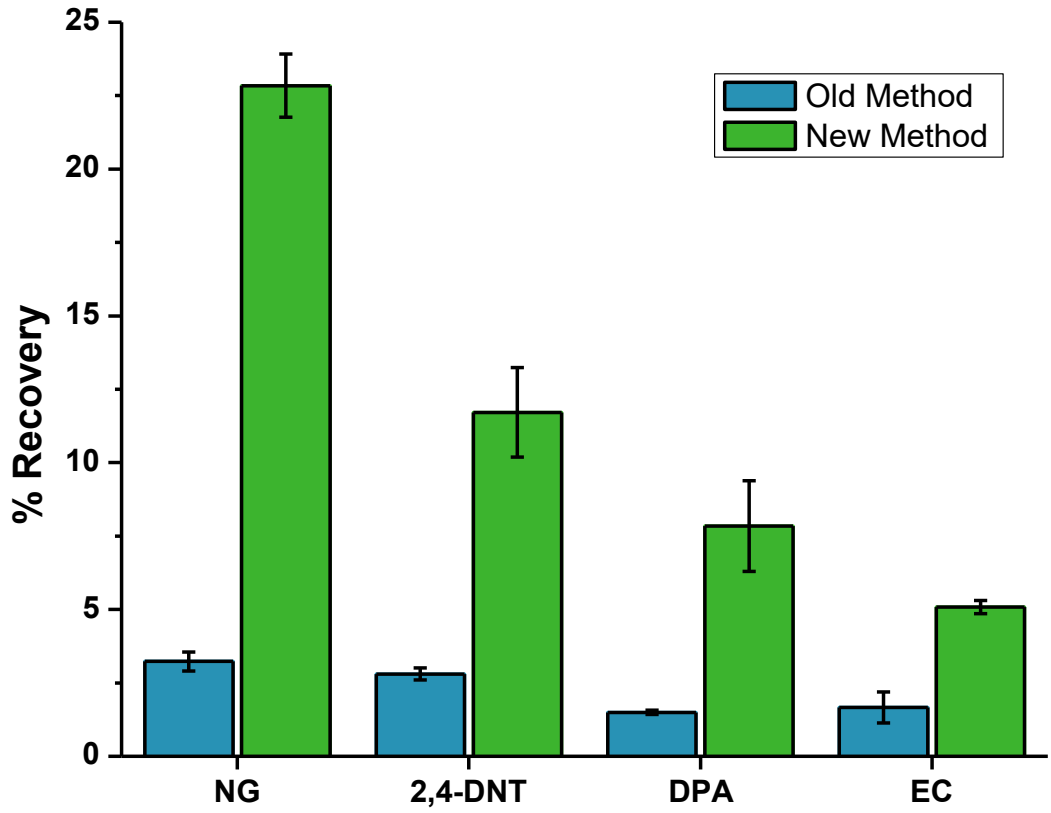


Figure 18. Comparison of extraction method parameters in 15 mL vials. Amount spiked: 50 ng of each compound on cotton swab moistened with DI water. Old method: Heated at 65°C for 20 minutes over hot plate, Tygon tubing, CMV at 20°C, ELF Pump at 1 L/min for 2 min. New method: Heated at 80°C for 20 minutes in water bath, Teflon tubing, CMV at 20°C, ELF pump at 1 L/min for 3 min. (n=3 replicates)

4.4.4. Cryofocusing Experiments for Vials

After optimization of the sampling parameters, cryofocusing was evaluated for further improvement of the extractions. Cooling the CMV was expected to improve

extraction recoveries of samples with low spiked masses. An experiment was performed with a 10 ng spike on the swabs in the vials with the optimized parameters. Surprisingly, application of cryofocusing resulted in a decrease in the recoveries for all compounds.

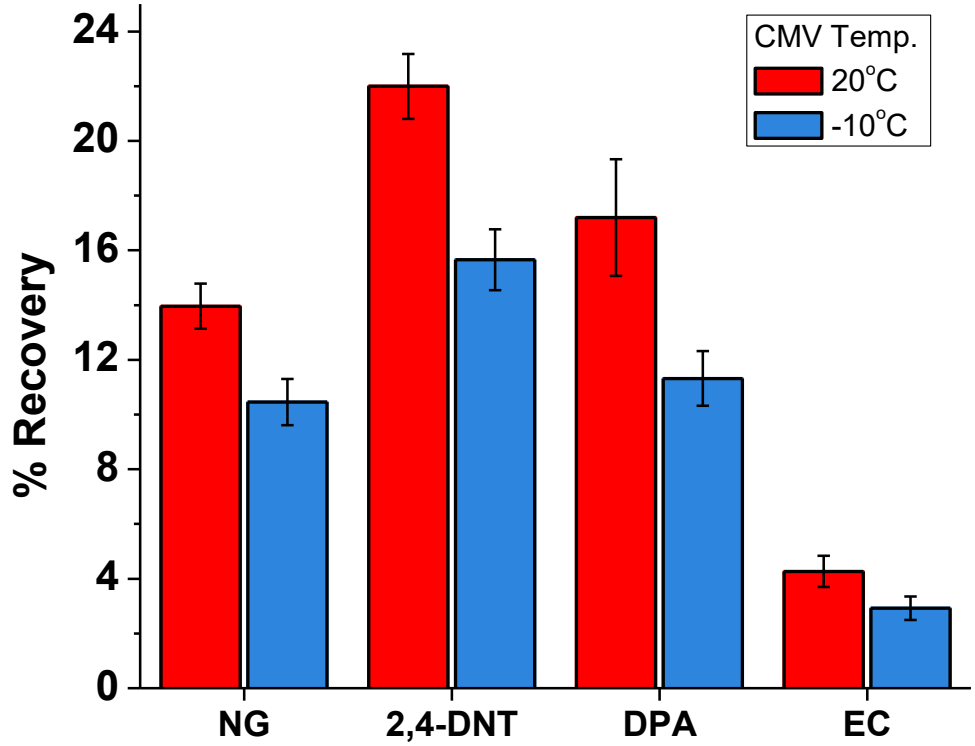


Figure 19. Evaluation of cryofocusing for 10 ng spike in 15mL vials on DI water moistened cotton swabs. Equilibrium time: 20 minutes. Heating temperature: 80°C. Flow rate: 1 L/min. Sampling time: 3 min. (n=3 replicates)

There were several explanations for the obtained results based on key observations regarding the cooler. First, when the cryofocused extraction was performed, there was buildup of condensation and moisture on the tubing inside the cold metal block. Also, visible water droplets were observed inside the CMV due to the low temperatures. Neither of these occurred when the CMV was maintained at room temperature. It was conjectured that compounds pumped from the vial headspace to the CMV were lost along the cooled tubing where there was a buildup of condensation. Therefore, it was uncertain whether the

cryofocusing actually did provide a benefit that was obscured by the condensed water negatively affecting the extraction.

4.5. Testing of Water Condensation

Different tests were performed to determine how to avoid condensation of water in the CMV and tubing. The action of only cooling the CMV and tubing without pumping air did not produce much condensation. However, when ambient air at room temperature was actively pumped through the system, both the tubing and CMV had moisture buildup. The longer the air was pumped, the more condensation was observed in the cooled CMV and tubing.

4.6. Backflash of GC Inlet

Another important factor to determine was if having water on the CMV affected the chromatography. Injection of small amounts of water was not predicted to negatively affect the GC column, which is a DB-5 MS 5% phenyl-methylpolysiloxane that is nonpolar, bonded, and cross-linked. These qualities make it suitable for injection of small amounts of water. Also, no differences in peak shape or retention time were observed for cryofocused samples.

Backflash in the GC inlet was another possible explanation for lower cryofocusing recoveries. Backflash occurs when the expansion volume of the solvent exceeds the volume capacity of the liner. Water has a very large expansion volume, which could potentially lead to sample loss if the analytes escape the liner. This was tested by spiking a known amount of each compound directly on either a pre-cooled or room temperature CMV. There was no apparent difference observed in peak height or areas, which indicated that the small

amount of condensation visible on the cold CMV did not affect the chromatographic analysis.

4.7. Experiments with Can Samples

4.7.1. Extraction of Smokeless Powders Containing NG in Cans

The containers were switched to the 1 L cans for further evaluation of the cryofocusing device. The 15 mL of headspace was potentially sampled too rapidly from the vials and did not provide adequate time for the cryofocusing to take effect. The 1 L cans have a much larger volume that is sampled gradually which may provide greater insight into the cryofocusing mechanism.

Cryofocusing was evaluated for enhanced sampling of the headspace of smokeless powders rather than standard solutions. This served as a test of only one compound at a time instead of all four. In the first experiment, 100 mg of double-based Hodgdon BLC2 rifle powder was weighed and placed directly on the bottom of several 1 L cans and sealed for an overnight incubation at room temperature. The lids were pre-punctured with red rubber sleeve septa to accommodate the headspace extraction. Separate cans were sampled at three different CMV temperatures to compare the effects.

An improvement was observed when sampling the vapors at 2.5°C and -10°C as seen in Figure 20. It was expected that -10°C would provide the highest recoveries. However, an observed increase in condensation at -10°C potentially affected the recovery. At 2.5°C, there was noticeably less condensation in the system. A new calibration curve was created with higher concentration solutions of NG (0-200 ppm) to quantify the amount recovered from the headspace. Evaluation of the results with an independent two-sample t-test indicated there was no statistically significant difference (with a p-value threshold of

0.05) between the three temperatures. However, enhanced recoveries with the Cryo-CMV may potentially be achieved by addressing the condensation buildup.

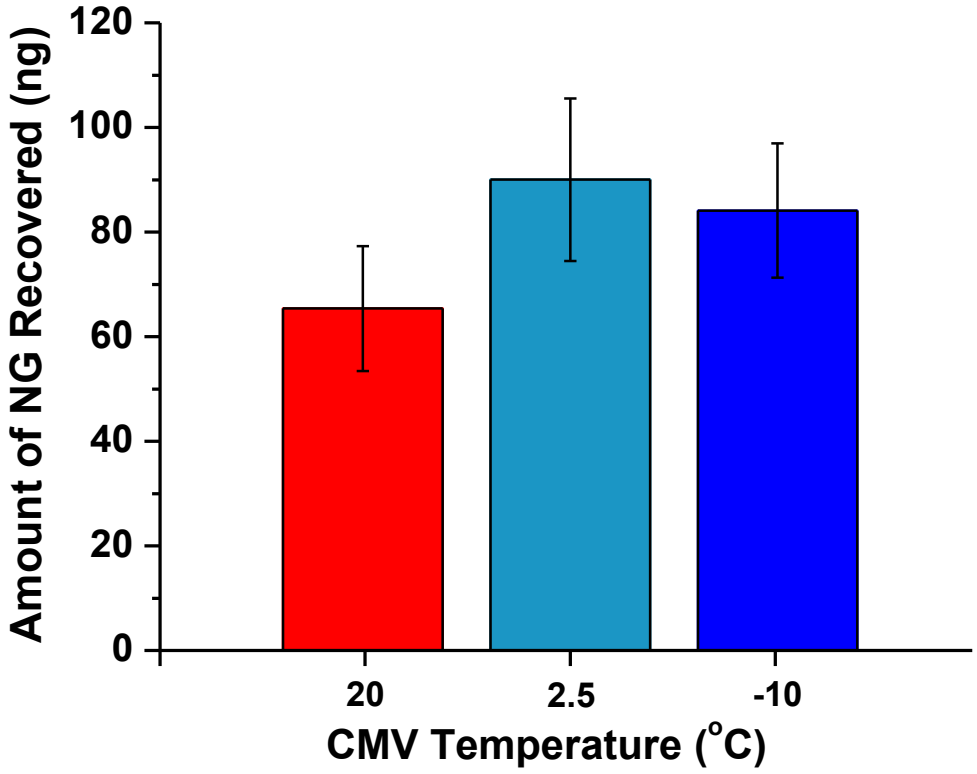


Figure 20. Cryofocusing for extraction of headspace of NG in Hodgdon BLC2 smokeless powder. 100 mg of smokeless powder stored in 1 L cans overnight at room temperature. Flow rate: 1 L/min. Extracted volume: 2 L. (n=3 replicates)

In order to test the effect of condensation, smokeless powder samples were incubated overnight with equal amounts of desiccant (Drierite) to remove any moisture. Although adding another substance may have decreased the amount of NG in the headspace, it was important to test the effect of removing the water buildup. Notably, when the extraction occurred, the tubing and CMV were completely dry. This resulted in an increase in the amount recovered at -10°C relative to 2.5°C in Figure 21. The apparent increase in recoveries for the cryofocused extraction shows that further improvements to the setup and overall optimization will provide benefits.

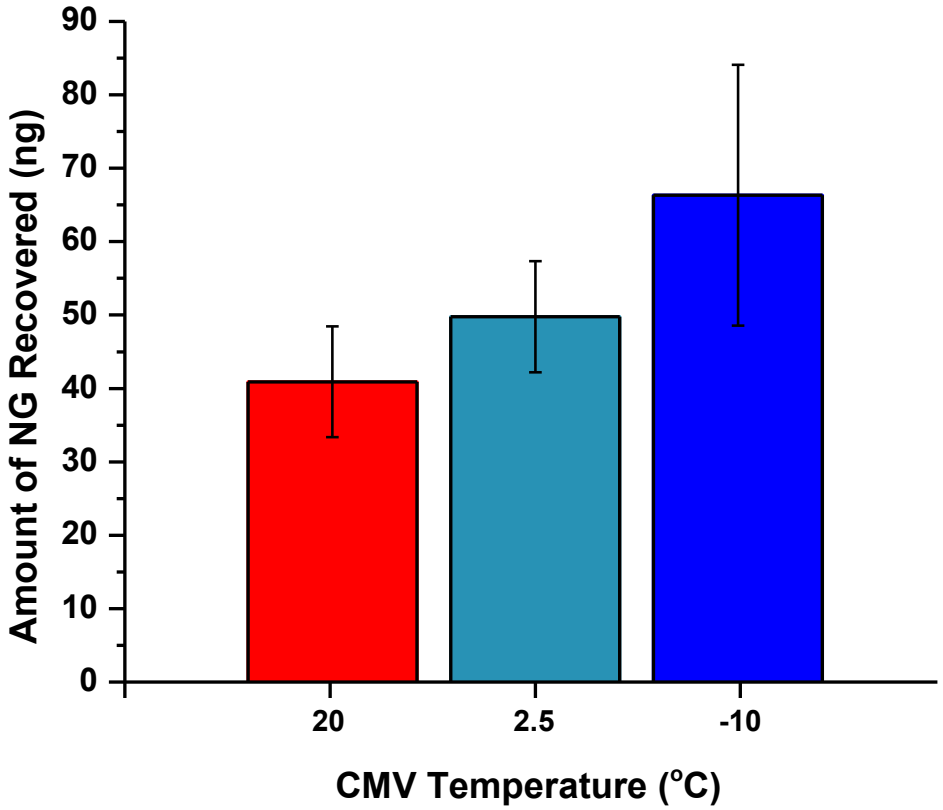


Figure 21. Extraction of dried smokeless powder containing NG at different CMV temperatures. 100 mg of Hodgdon BLC2 stored in 1 L cans overnight with desiccant at room temperature. Flow rate: 1 L/min. Extracted volume: 2 L. (n=3 replicates)

4.7.2. Extraction of Smokeless Powders Containing 2,4-DNT in Cans

Additional experiments were conducted to test whether cryofocusing would improve the recovery of smokeless powders containing 2,4-DNT instead of NG. An equal amount (100 mg) of Hodgdon H322 rifle powder was weighed and placed in 1 L cans and sealed for an overnight equilibrium. They were extracted at the same temperatures as the NG containing smokeless powders. However, there was no observed difference between the CMV temperatures for extraction of 2,4-DNT, which may be attributed to its higher boiling point. The integrated peak areas were not quantified since they were above the calibration scale, although they still enabled relative comparisons in Figure 22.

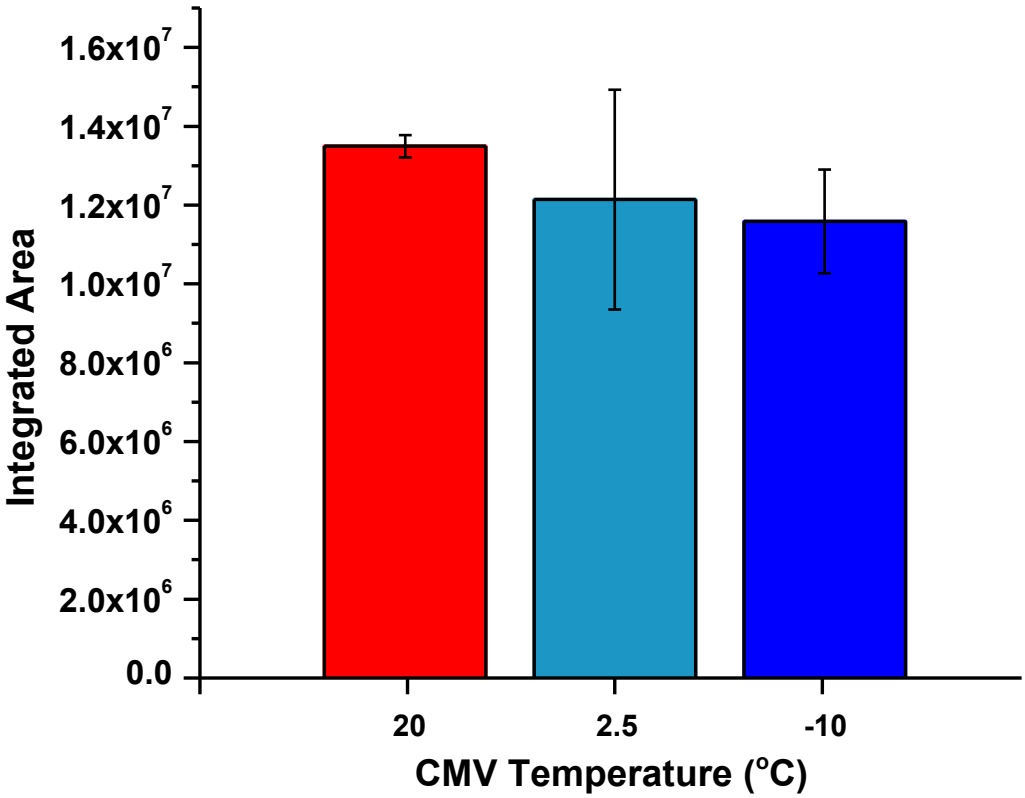


Figure 22. Extraction of Hodgdon H322 smokeless powder containing 2,4-DNT. 100 mg of smokeless powder incubated in 1 L cans overnight at room temperature. Flow rate: 1 L/min. Extracted volume: 2 L. (n=3 replicates)

4.7.3. Comparison of CMV Temperatures in 1 L Cans

The research shifted focus toward extraction of OGSR compounds in the 1 L cans. The larger volume of headspace was expected to be extracted gradually from the 1 L cans versus the 15 mL vials. Previous research by Fan indicated that at least a 500 ng spike of these compounds directly in the cans was required for detection in the headspace. [69] The reported calibration curves were generated from 500-1500 ng; therefore, 1000 ng was selected as an appropriate intermediate amount to spike directly in the cans for evaluation of cryofocusing. The expected recoveries were between 1-2% for these compounds using the same conditions as described by Fan. [69] Experimental conditions were adopted from

prior optimization experiments, which indicated 10 minutes of equilibrium time was ideal to avoid a decrease in recovery for DPA.

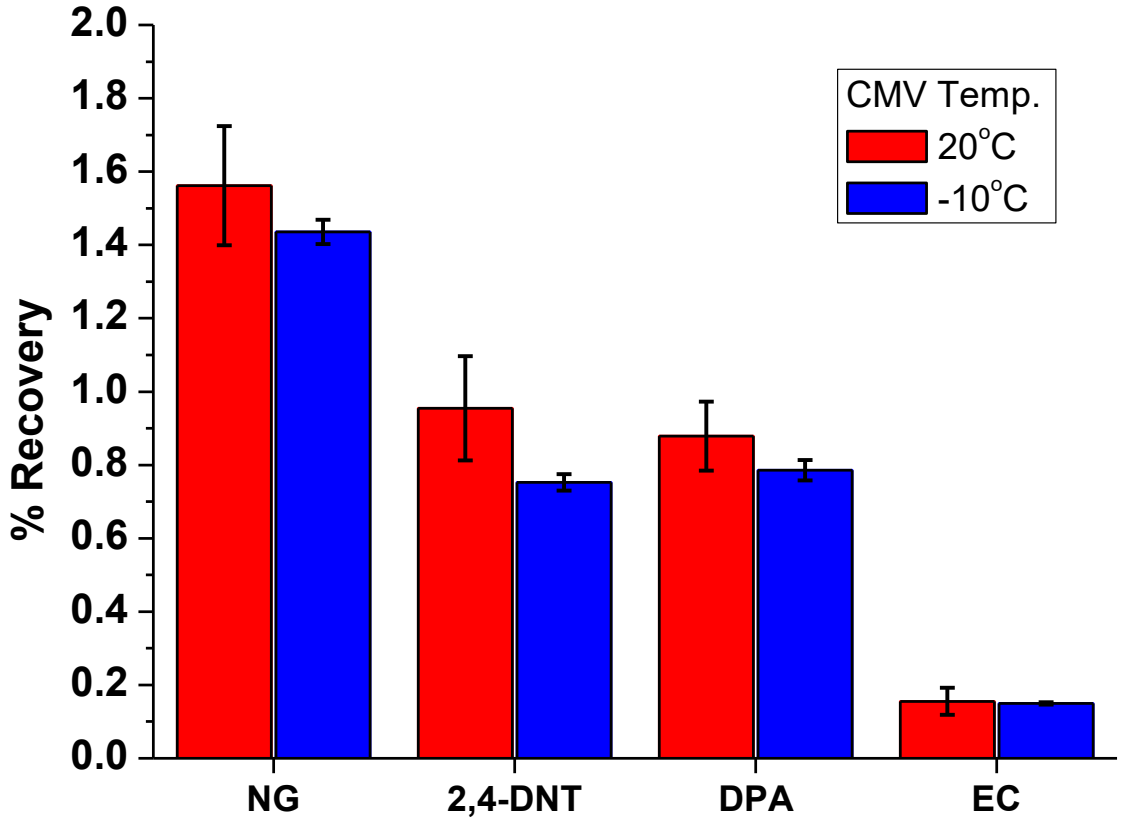


Figure 23. Cryofocusing for extraction of OGSR compounds in 1 L cans. Equilibrium time: 10 min @ 20°C. Tygon tubing. ELF pump @ 1 L/min for 2 min. (n=3 replicates)

Figure 23 shows that cryofocusing resulted in slightly lower recoveries relative to room temperature. Higher recoveries were obtained for the more volatile compounds; EC was the least volatile with the lowest recoveries. Once again, condensation buildup was observed with the cryofocused extraction. Overall, the recoveries were very low in the cans and spiking the solutions directly on the swabs would not be feasible at these concentrations.

4.7.4. Breakthrough Experiment in 1 L Cans

Investigation of possible breakthrough of compounds was studied to understand the mechanism of cryofocusing. Breakthrough occurs when compounds are pumped from the air and escape out the other end of the tube due to a lack of retention by the sorbent. Cryofocusing is expected to prevent breakthrough by trapping analytes on the CMV. This was tested by connecting two CMVs in series and analyzing both the front and back CMVs. The front CMV encounters the sampled air first, and the back CMV is directly behind it to check for any escaping analytes. The amount recovered for each CMV was computed and the breakthrough percentage was calculated in Figure 24 and Table 3, respectively.

$$Breakthrough\ \% = \frac{Amount\ Recovered\ (ng)\ on\ Back\ CMV}{Total\ Amount\ Recovered\ (ng)\ on\ Front{+}Back\ CMV}\ X\ 100\% \qquad (5)$$

Table 3: Calculated average breakthrough % for each compound in Figure 24 at different CMV temperatures.

	Average Breakthrough %	
Compound	20°C	-10°C
NG	24	25
2,4-DNT	28	30
DPA	21	24
EC	23	30

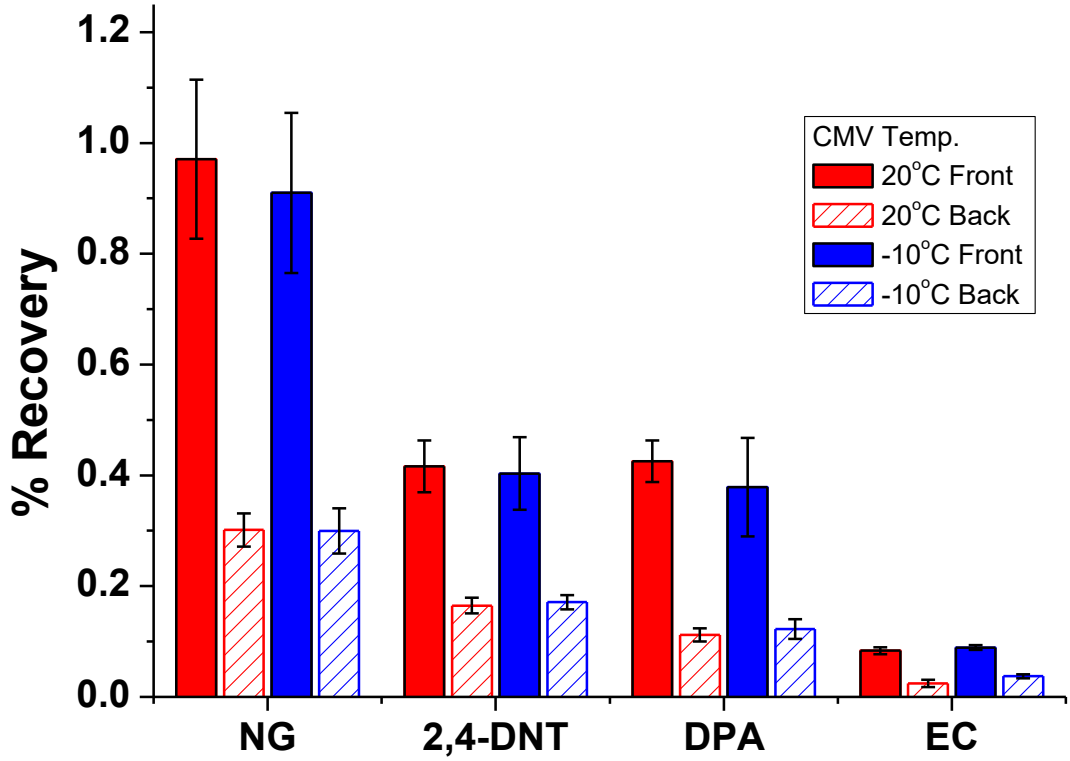


Figure 24. Determination of breakthrough at different CMV temperatures. Amount spiked: 1000 ng of each compound in 1 L cans. CMVs connected in series; 10 minute equilibrium at 21°C; ELF pump @ 1 L/min for 2 min extraction. (n=3 replicates)

The experiment shows there is no apparent difference in breakthrough when comparing these two CMV extraction temperatures. Cryofocusing was anticipated to reduce the breakthrough of analytes and increase the amount recovered as a result. These results indicate that the CMV already performs effectively at extracting the semi-volatile GSR compounds under these experimental conditions. In light of these findings, the focus was directed towards other parameters to determine their influence on the cryofocusing.

4.7.5. Flow Rate Comparison in 1 L Cans

The flow rate of the pump is a crucial parameter that required further investigation. In the case of the semi-volatile OGSR compounds, it was found that higher flow rates produce higher recoveries. Lower flow rates were preferred for more volatile BTEX

compounds. [69] An experiment was performed to determine if the flow rate also had an impact on the cryofocusing. The total extracted volume was fixed and the required sampling time was calculated with the equation below. The maximum attainable stable flow rate through the CMV was 1.3 L/min. The total extracted volume was 2 L for all flow rates.

$$Extracted\ volume\ (L) = Flow\ rate\ \left(\frac{L}{minute}\right)\ X\ Sampling\ Time\ (minutes) \quad (6)$$

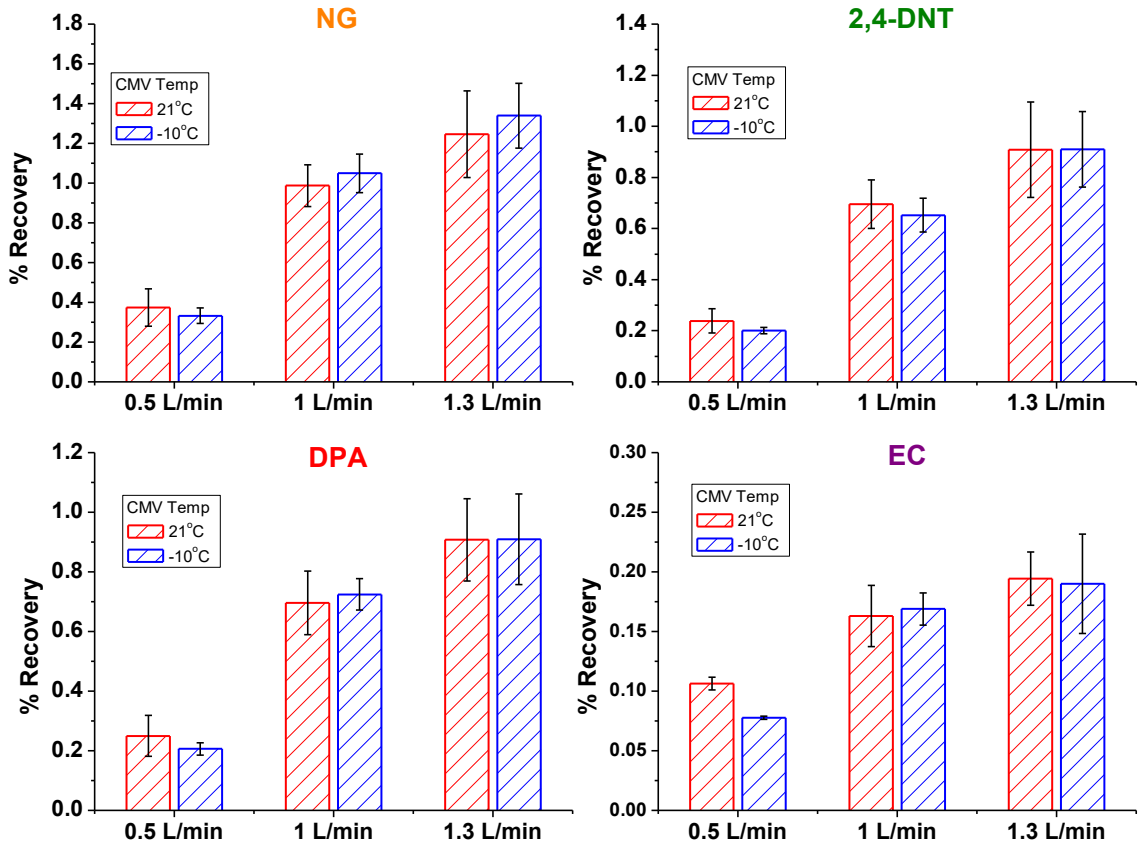


Figure 25. Flow rate comparison. 1000ng spike of each compound in 1 L cans. 10 minute equilibrium at 21°C. ELF pump at specified flow rates. 2 L total extracted volume. (n=3 replicates)

Clearly, Figure 25 shows that the flow rate had a major impact on the recoveries. Higher flow rates were beneficial for these semi-volatile compounds and greatly increased the amount recovered. This is the opposite case when compared to the BTEX compounds which are more volatile and suffer a breakthrough at high flow rates. [10] However, cryofocusing combined with increasing the flow rate did not appear to provide substantial enhancements over room temperature extractions.

4.7.6. Temperature Curve for 1 L Cans

Further investigation of the effect of the cryofocusing was performed by programming the Peltier cooler to a range of different temperatures for the extraction. The optimized flow rate of 1.2 L/min was selected for all extractions in this experiment. Higher flow rates resulted in pumping more moisture onto the CMV, so 1 L/min was selected for future experiments. No apparent difference was observed at these temperatures in Figure 26.

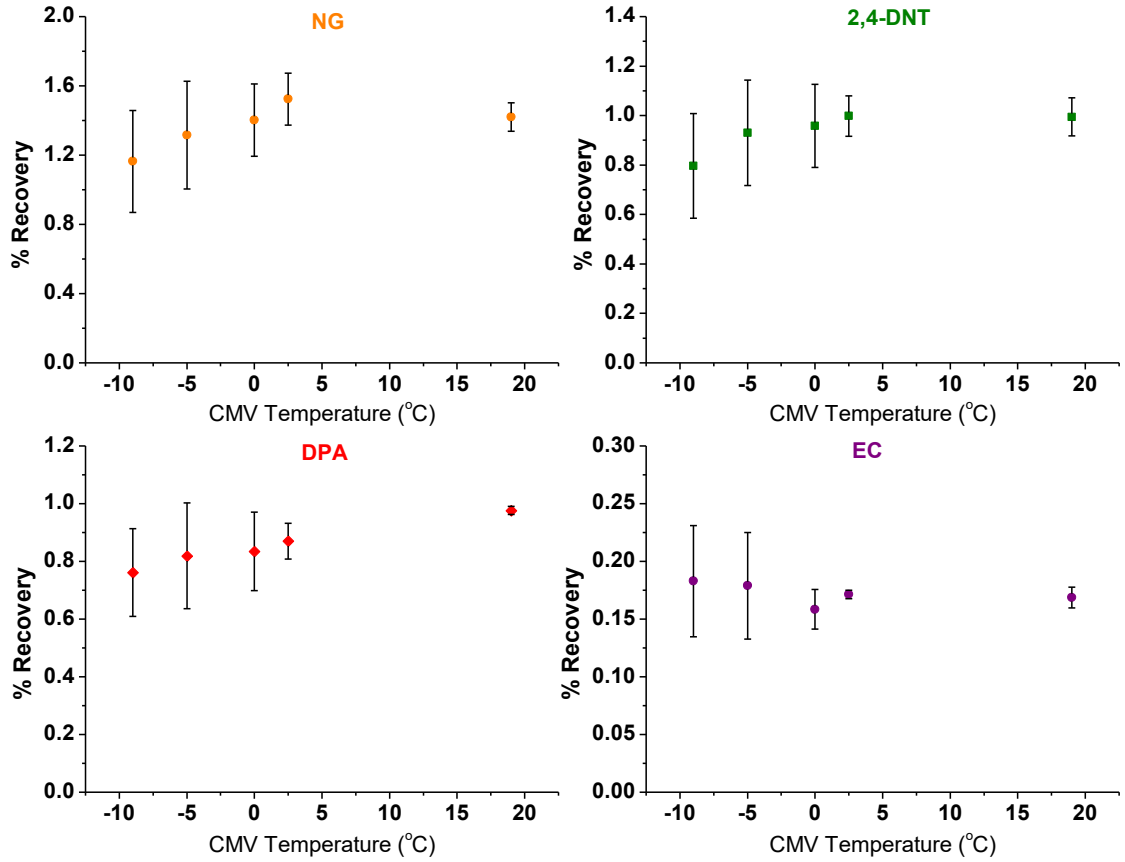


Figure 26. CMV temperature comparison for extraction of OGSR compounds. 1000 ng in 1 L can; 10 min equilibrium at 20°C; 1.2 L/min flow rate, 2 L total extracted volume (n=3 replicates)

4.7.7. Effect of Tubing Type

The type of tubing was switched from Tygon to Teflon in order to improve the low recoveries observed in the cans. The SIM peaks are compared in Figure 27. Teflon provided a lower background and greater temperature resistance for future heating experiments with the cans. Teflon provided significantly higher recoveries when all other factors were kept constant in the setup. This indicated that the transfer line connecting the CMV to the container was an important factor.

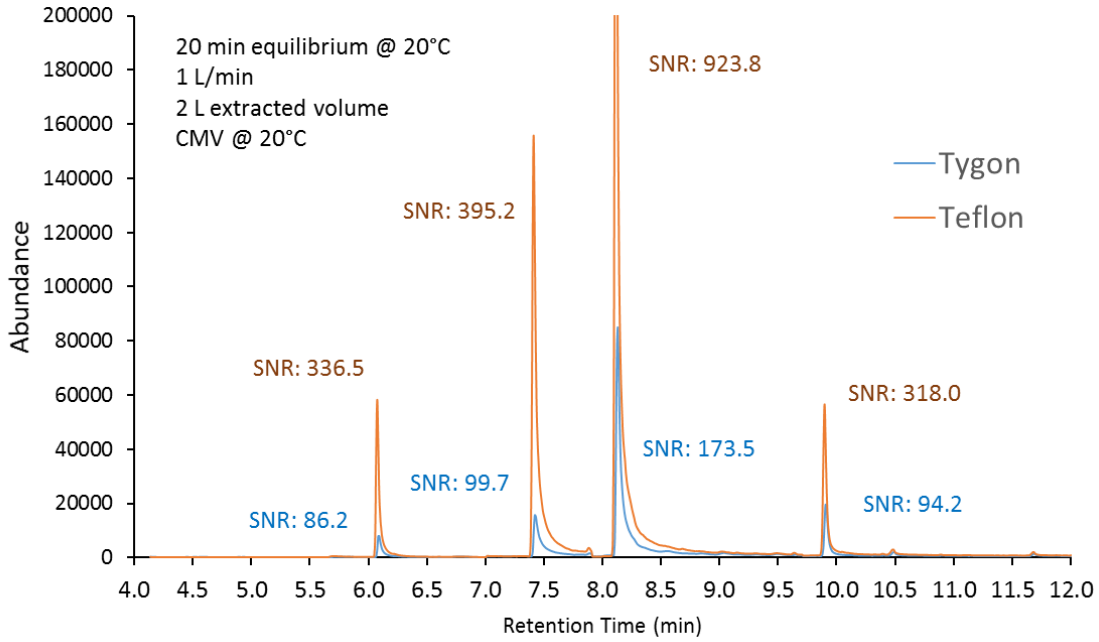


Figure 27. SIM chromatogram showing improvement when switching to Teflon tubing for the transfer line to the CMV versus the previously used Tygon tubing. Signal to noise ratios are compared for each compound. Amount spiked: 1000 ng in 1 L can.

4.7.8. Heating Temperature Optimization for 1 L Cans

As a result of the low recoveries observed in the prior experiments with the 1 L cans, it was deemed necessary to heat the container to increase the amount in the headspace. The solutions were initially spiked and sealed in the cans for 10 minutes at room temperature, then transferred to the heater for an additional 10 minutes. Immediately heating the can after spiking the solution evaporated the solvent too rapidly which negatively affected the recoveries. A temperature optimization test revealed the ideal can temperature for these compounds in Figure 28.

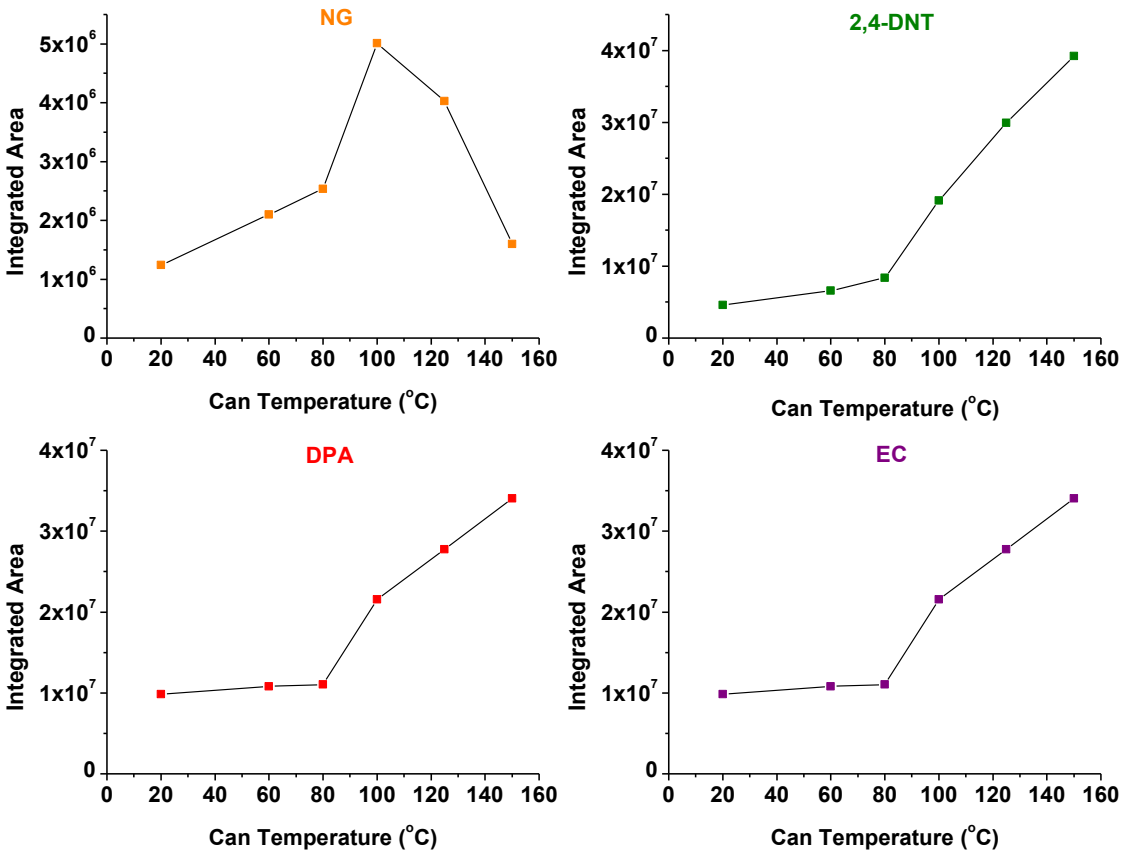


Figure 28. Optimization of heating temperature. Amount spiked: 1000 ng of each compound in 1 L cans. Equilibrium time: 10 minutes at room temperature, then 10 minutes at heated temperature. CMV temperature: 20°C. Extracted with ELF pump. Flow rate: 1 L/min. Extracted volume: 2 L.

An improvement was seen as the temperature increased for DPA, 2,4-DNT, and EC, especially after heating above 80°C. However, for NG, after 100°C, there was a decrease in the amount detected. This phenomenon was expected and attributed to decomposition of NG at elevated temperatures as previously noted. Thus, the optimum temperature for extraction in the cans is at around 100°C to prevent NG loss.

4.7.9. Comparison of CMV Temperature in Heated Cans

After determining the optimal temperature, cryofocusing was tested again to see if there is an improvement when sampling heated vapors. The experiment was performed

with the can at 100°C while maintaining the other conditions to test the effect of heating the sample. Despite the improvement in the recoveries, there was no difference observed when the cryofocusing was applied for the extraction of the heated vapors.

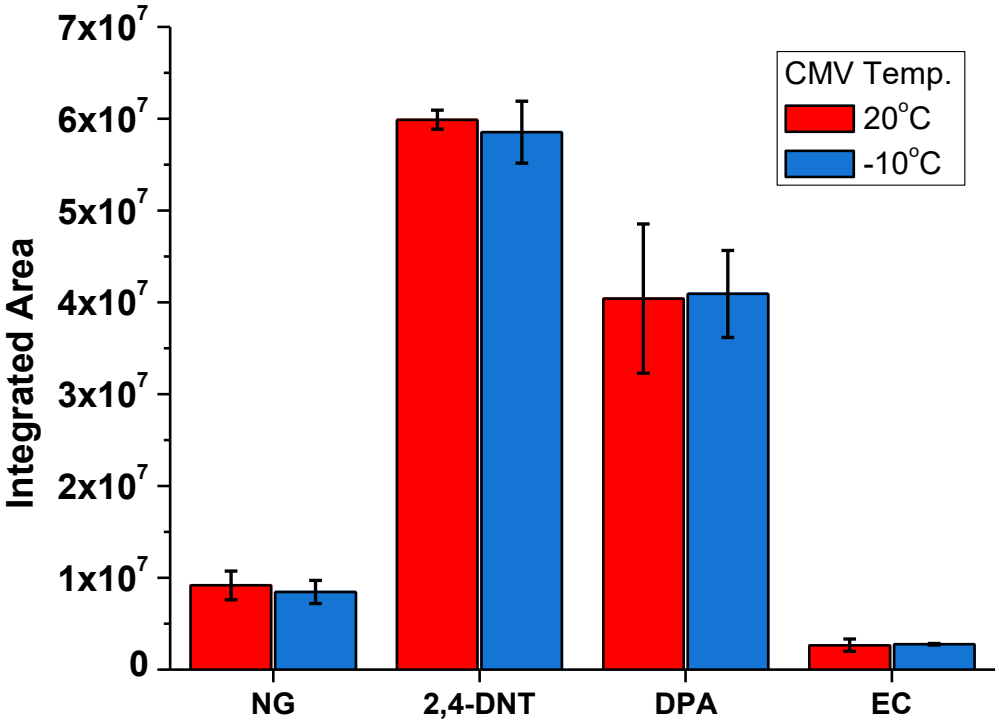


Figure 29. Cryofocusing for extraction of heated cans. Amount spiked: 1000 ng in 1 L cans. Equilibrium time: 10 minutes at 20°C, then 10 minutes at 100°C. Flow rate: 1 L/min. Extracted volume: 2 L. (n=3 replicates)

A new hypothesis was formed following the important conclusions reached by this set of experiments. First, the transfer line from the container to the CMV greatly influences the recoveries. Second, heating the can combined with application of cryofocusing did not result in an improvement. However, the transfer line from the can to the CMV was not heated. It was theorized that a drop in temperature from the heated can to the transfer line at room temperature affected the sampling of the heated vapors. The vapors could condense

along the tubing, essentially achieving the purpose of the Peltier cooler before the analytes actually reach the CMV. Heating the transfer line was considered as a potential solution to this issue.

4.8. Heated Transfer Line to CMV in Cans

The transfer line from the CMV to the container was heated to prevent condensation of compounds prior to reaching the cryofocusing module. The internal temperature of the tubing was measured with a thermocouple at approximately 60°C when heated externally with a hair dryer. The cans were heated to 70°C rather than the optimal 100°C for this experiment. Fluctuations in the can temperature of about 2-3°C occurred when the transfer line was heated, which had a larger effect in the more sensitive heating region at 100°C based on the previously obtained temperature curve.

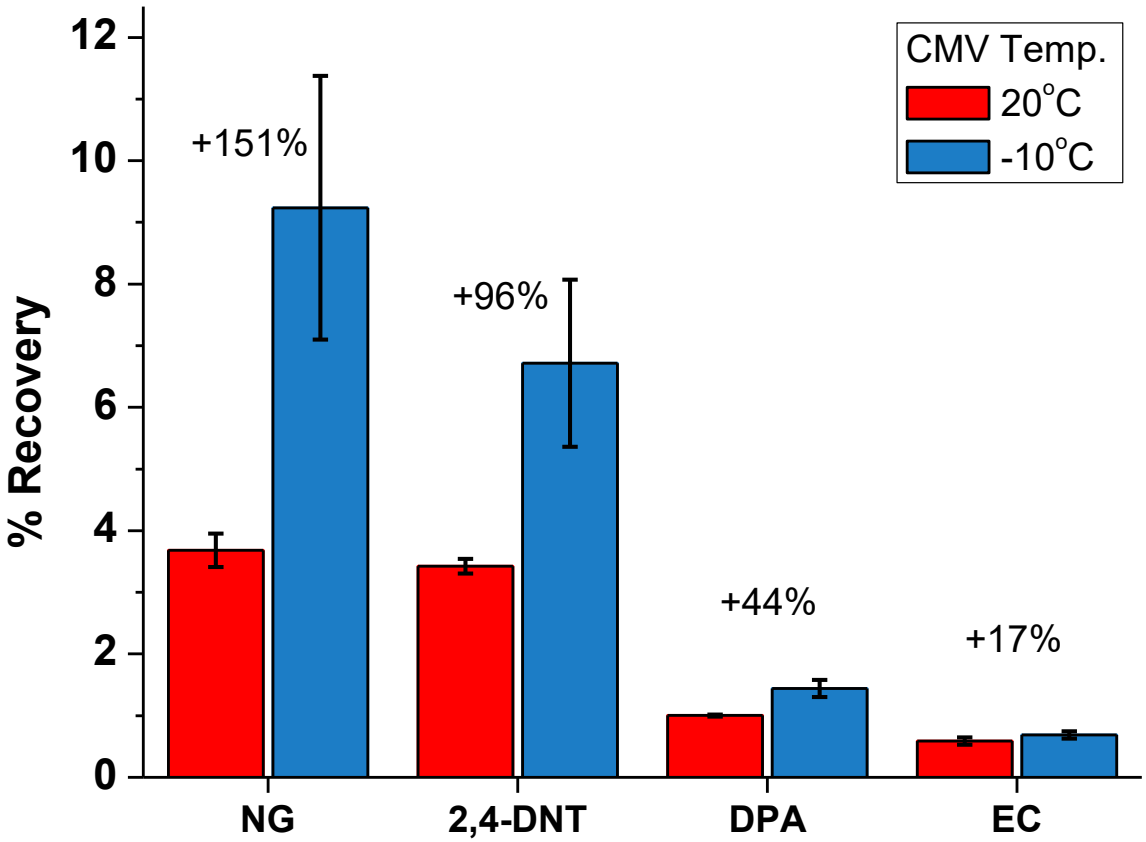


Figure 30. Heated transfer line experiment with cryofocusing. Amount spiked: 1000 ng in 1 L cans. Equilibrium time: 10 min at 20 °C then 10 min. at 75°C. Flow rate: 1 L/min. Extracted volume: 2 L. (n=3 replicates)

The combination of heating the transfer line with cryofocusing resulted in a substantial improvement in recoveries. For the first time, an increase in recoveries for all compounds was observed when cryofocusing was applied in conjunction with heating the transfer line. A significant difference in recoveries between 20°C and -10°C was found for NG, 2,4-DNT, and DPA using an independent two-sample t-test (p-value threshold = 0.05). Moreover, this was the first time that no condensation of water was observed on the tubing or the CMV which is attributed to heating of the transfer line.

4.9. Heated Transfer Line to CMV in Vials

Since heating the transfer line was successful for the can samples, the focus was returned to the vial samples. An experiment was conducted to compare the effect of cryofocusing with and without heating the transfer line. Once again, no condensation was observed on the CMV during or after the extraction when heating the transfer line. Cryofocusing also provided an improvement in the vials by heating the transfer line. The overall recoveries improved when the transfer line was heated as well as seen in Figure 31. A significant difference in recoveries between 20°C and -10°C (with heated transfer line) for NG and 2,4-DNT was found; results were evaluated with an independent two-sample t-test (p-value threshold=0.05).

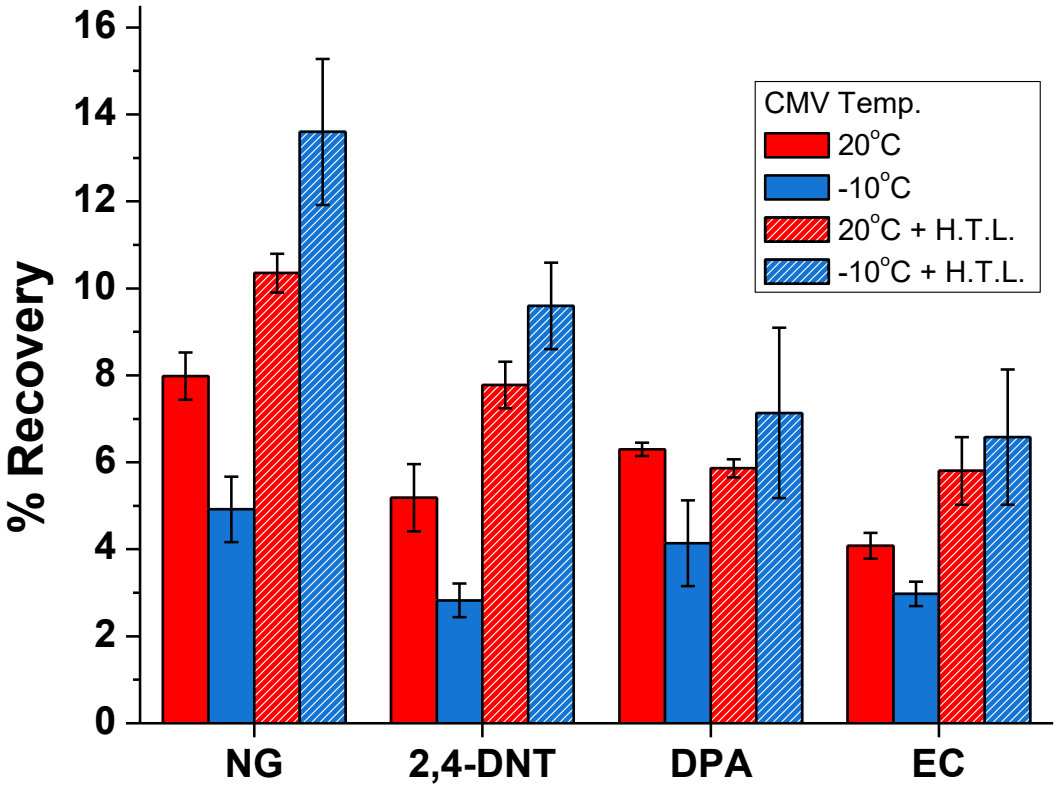


Figure 31. Comparison of cryofocusing for heated (H.T.L.) and non-heated transfer line in vials. Amount spiked: 100 ng on cotton swab moistened with DI water. Equilibrium time: 20 minutes at 75°C. Flow rate: 1 L/min. Extracted volume: 3 L. (n=3 replicates)

4.10. Air Sampling Experiments

The sample collection methods described previously were intended for swabbing of the shooter's hands. Another motivation of this research was testing whether a portable air sampling pump that accommodates the CMV without tubing is capable of collecting OGSR volatiles. If successful, it would bypass the swabbing and headspace extraction steps, enabling the CMV to capture the OGSR volatiles followed by direct analysis with GC-MS.

A preliminary evaluation of the sampling pumps was conducted by mimicking field samples. For this purpose, 10 µg of the OGSR compounds was spiked evenly across either a nitrile glove or square (4 X 4 inches) section of cotton fabric. The glove and fabric were placed inside paper bags and tied at the ends with a rubber band to simulate real-world scenarios. In situations where a suspect has potentially handled explosives, law enforcement will cover the individual's hands with a paper bag in a similar manner to preserve any traces of volatile compounds. [70] The samples were placed in the bags for 20 minutes before the extraction to build up the concentration in the headspace. Extractions were performed by puncturing a round hole in the paper bag and pumping with the CMV placed directly above the hole. Blank air samples were collected from the paper bag with the blank cloth and glove before spiking the compounds.

The OGSR compounds were not detected on the mock glove sample, although it may be attributed to poor evaporation of the solvent off the glove's nitrile material. The extraction of the cloth sample shows clear detection of all four targeted compounds. Thus, the handheld air sampler shows viability for sampling of OGSR residues on sections of clothing from a suspected shooter.

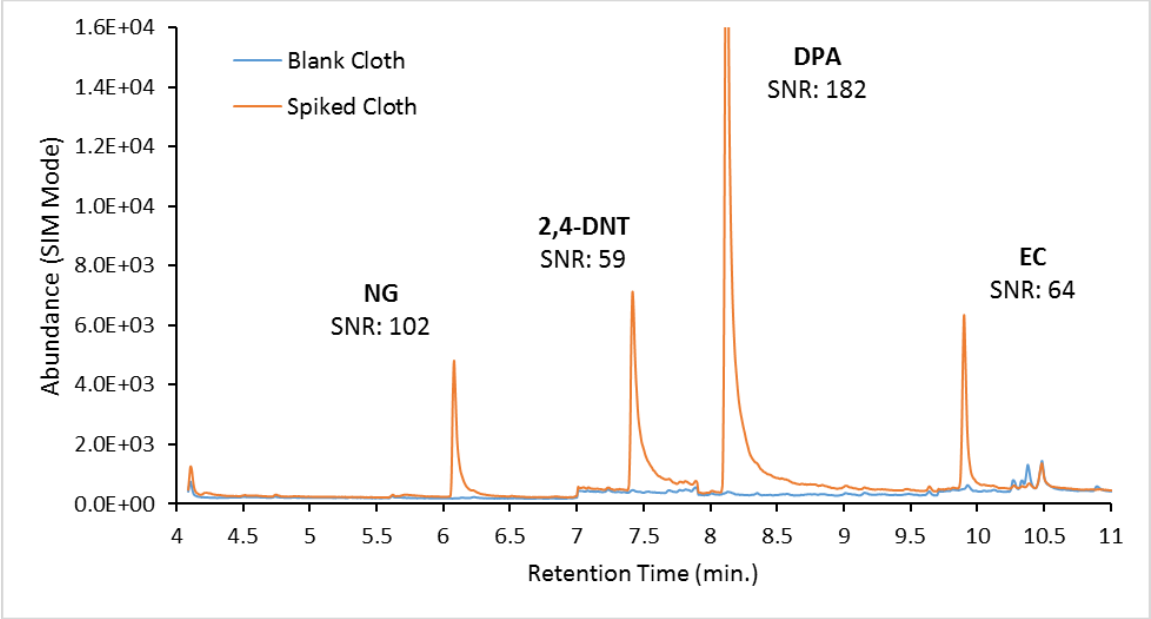


Figure 32. SIM chromatogram of extraction of headspace of a closed paper bag containing a cotton fabric spiked with 10 µg of OGSR compounds. Equilibrium time: 20 minutes. Sampled volume: 3 L.

4.11. Preliminary Field Sampling Experiments

Initial field sampling experiments were conducted prior to the optimization of the parameters. The goal was to determine whether the collection and extraction method was capable of detecting as few as one shot on the hands of shooters. Additionally, both the vials and cans were compared to determine which container type is preferred for field sampling. Any complications or difficulties encountered were also noted for future improvement.

Two police officers were asked to volunteer for collection of hand swab samples. Sample collection was performed according to the protocol described in chapter 3.4. Blank hand swabs were obtained prior to handling of the firearm. Both officers fired shots from a 9mm handgun with Winchester full metal jacket ammunition. The safety data sheet for the cartridges lists nitroglycerin, ethyl centralite, and diphenylamine, but not 2,4-

dinitrotoluene in the smokeless powder. The officers were asked to fire a series of consecutive shots from one to five. A hand swab was collected from the right hand that held the firearm after each firing event. Swabs were immediately stored in either the 15 mL vials or 1 L cans at ambient temperature. In the vial samples, the septa in the lids were punctured to insert a CMV to facilitate immediate static headspace extraction. The CMV and vial were then sealed with aluminum foil to prevent loss of volatiles. In the can samples, pre-punctured holes on the lids were sealed with rubber sleeve septa without inserting any CMV devices.

The samples were analyzed in the laboratory within 48 hours by dynamic headspace extraction with the Escort ELF Pump. The samples from the shooters were analyzed with different sets of parameters to determine which would perform the best. The vial samples contained the CMVs in the lid of the septa for combined static and dynamic extraction, so they could not be inserted in the Peltier cooler. The ELF pump was directly connected to the CMV with a plastic adapter for the vial samples. The can samples were extracted with the CMV under cryofocusing conditions in the Peltier cooler. Containers were heated to approximately 65°C by placing them directly above a hot plate.

Data analysis was performed by evaluating the chromatograms and mass spectra for the full scan, SIM, and μECD modes. The four compounds of interest were searched by their retention times and characteristic fragment ions previously listed in Table 2. Extracted ion chromatograms were obtained in full scan mode for each compound of interest with the aforementioned fragment ions. A compound was considered present in the sample if the peak's signal to noise ratio was greater than 10 as determined by the Chemstation software. The field sampling results are presented in Table 4.

Table 4. Preliminary field sampling results for hand swabs of shooters.

Shooter 1	*All samples extracted with ELF Pump* Flow rate: 1 L/min; Extracted volume: 2 L				Compound Detected? If yes=Y (SNR >10)									
					NG			2,4-DNT			DPA		EC	
Sample	Can/Vial	Container Temperature	CMV Used	CMV Temp.	Full Scan	SIM	µECD	Full Scan	SIM	µECD	Full Scan	SIM	Full Scan	SIM
Hand Blank	Vial	21°C (Room)	1X-S1	21°C										
1 Shot	Vial	21°C (Room)	1X-S1	21°C										
2 Shot	Vial	21°C (Room)	2X-S1	21°C										
3 Shot	Vial	21°C (Room)	3X-S1	21°C										
4 Shot	Vial	21°C (Room)	4X-S1	21°C										
5 Shot	Vial	21°C (Room)	5X-S1	21°C										
Can Blank	Can	21°C (Room)	CMV #4	-6°C										
1 Shot	Can	21°C (Room)	CMV #4	-6°C										
2 Shot	Can	21°C (Room)	CMV #4	-6°C										
3 Shot	Can	21°C (Room)	CMV #4	-6°C										
4 Shot	Can	21°C (Room)	CMV #4	-6°C										
5 Shot	Can	21°C (Room)	CMV #4	-6°C										
Shooter 2	*All samples extracted with ELF Pump* Flow rate: 1 L/min; Extracted volume: 2 L				Compound Detected? If yes=Y (SNR > 10)									
					NG			2,4-DNT			DPA		EC	
Sample	Can/Vial	Container Temperature	CMV Used	CMV Temp.	Full Scan	SIM	µECD	Full Scan	SIM	µECD	Full Scan	SIM	Full Scan	SIM
Can Blank	Can	60-70 °C (Heated)	CMV #4	-6°C										
1 Shot	Can	60-70 °C (Heated)	CMV #4	-6°C										
2 Shot	Can	60-70 °C (Heated)	CMV #4	-6°C										
3 Shot	Can	60-70 °C (Heated)	CMV #4	-6°C										
4 Shot	Can	60-70 °C (Heated)	CMV #4	-6°C										
5 Shot	Can	60-70 °C (Heated)	CMV #4	-6°C										
Hand Blank	Vial	21°C (Room)	SB2	21°C										
1 Shot	Vial	21°C (Room)	1X-S2	21°C										
2 Shot	Vial	21°C (Room)	2X-S2	21°C										
3 Shot	Vial	21°C (Room)	3X-S2	21°C										
4 Shot	Vial	21°C (Room)	4X-S2	21°C	Y	Y	Y				Y	Y		
5 Shot	Vial	21°C (Room)	5X-S2	21°C	Y	Y	Y				Y	Y		

Several conclusions were drawn from the preliminary field sampling experiments. First, the OGSR compounds were not detected in any of the can samples which was attributed to the large container volume. Vials are preferred for their smaller volume that increases the concentration in the headspace as well as ease of transport and storage. Also, both NG and DPA were detected after 4 shots in the vials using static and dynamic extraction at room temperature. Heating the vials is necessary to detect OGSR from fewer shots. Additionally, ethyl centralite was not detected in any of the samples due to its low vapor pressure [9]; heating the container may address this issue. Furthermore, placing the CMV directly in the septa of the vial for static extraction is undesirable for field sampling. The samples should be tightly sealed with the septa intact to avoid loss of volatile compounds and prevent potential contamination. The method development and optimization in this research have addressed these issues for future field sampling experiments.

4.11.1. Sampling of Non-shooters

Six individuals at FIU who did not handle a firearm were asked for hand swab samples collected in a similar manner. The purpose was to identify any potential contaminants or interferences. No OGSR compounds were detected on any of the samples. Although the chromatograms had a high background, it was later attributed to the cotton swabs' plastic handles. A switch to cotton swabs with wood handles greatly lowered the background.

4.12. Results and Discussion

The final optimized parameters for OGSR extraction of hand swab samples in vials consist of heating to 80°C for 20 minutes and extracting 3 L of headspace at a 1 L/min flow

rate. The vial samples provided higher recoveries and are preferred over the cans for OGSR analysis. An improved setup with a water bath for consistent heating and needles for puncturing the septa for headspace extraction provided an increase in the recoveries.

A custom-built handheld air sampling pump connected directly to the CMV showed promising results for extracting OGSR spiked on a cotton fabric. Clothing is typically sampled by vacuum or tape lifting which requires solvent extraction. The handheld pump featured rapid sampling in just 3 minutes followed immediately by thermal desorption of the CMV in the injection port. Although the primary focus of this research was hand swab samples, the direct sampling technique with the handheld pump displayed great potential thanks to its simplicity.

The various aforementioned experiments provide important conclusions for cryo-CMV-GC-MS of OGSR compounds. The vapor pressure of the compounds was an important parameter. Cryofocusing increased the recovery of extractions of smokeless powders containing NG, but not 2,4-DNT due to its lower volatility. The semi-volatile nature of the OGSR compounds led to many challenges not encountered with VOCs in air. Semi-volatile compounds tend to form aerosols and droplets due to their low vapor pressures. [71] High sampling flow rates are preferred for CMV extractions of semi-volatile compounds. Condensation of water on the tubing and CMV were an issue because of the required high flow rates. These issues were overcome when the transfer line from the CMV to the container was heated which provided an increase in recoveries.

5. CONCLUSIONS

5.1. Significance of Results

The results of this study have several implications for CMV-GC-MS as a comprehensive method for OGSR analysis. Method development and optimization experiments provided many improvements. Investigation of cryofocusing for semi-volatile OGSR revealed that the CMV already performs effectively for extraction of these compounds at room temperature. Cryofocusing shows greater utility for more volatile compounds because of their tendency to have breakthrough when sampling.

This research also explored solutions to potential challenges encountered when working with the Peltier cooler. Buildup of condensation was an issue that was mostly corrected by heating the transfer line. Additional water management techniques include flushing the CMV with a gentle stream of nitrogen after the cold extraction. A reverse flow of an inert gas is often applied to remove moisture from commercial sorbent tube samplers. [72]

5.2. Future Work

Further improvements to the sampling and extraction method are desirable to provide practicality. Substitution of water as a solvent for hand sampling with ethanol or isopropanol may increase the amount of OGSR collected. Adding more sufficiently volatile target compounds such as 2,6-dinitrotoluene or dibutyl phthalate can increase the specificity of the technique. [5] However, the GC method may require modifications to the temperature program, inlet temperature, or column length to facilitate additional compounds. Different types of tubing are worth testing as well since it had a significant

effect. Also, a heating block designed for headspace vials can replace the water bath. A rope heater that wraps around the tubing can provide heating of the transfer line. The dynamic headspace extraction can also be performed with dry nitrogen flowing into the vial as opposed to ambient air.

Suggested future experiments involve testing the field sampling protocol. Once the method is finalized, creating a headspace calibration curve with the optimized parameters will provide the ability to calculate the amounts recovered from the field samples. Also, the stability of mock field samples requires evaluation over a range of time intervals. Validation studies will determine the associated false positive and false negative rates.

Another application of cryofocusing worth investigating is extraction of ignitable liquid residues in fire debris samples. The current ASTM methods for fire debris analysis recommend static headspace sampling with a SPME fiber and subsequent GC-MS analysis. A wide range of volatile and semi-volatile compounds are present in gasoline and diesel fuel. The extraction procedures described in this study for the OGSR compounds are adaptable to fire debris analysis. The 1 L cans used in this study are the same containers typically used for fire debris samples. The Cryo-CMV-GC-MS technique is expected to provide higher recoveries than the current static headspace technique for the ignitable liquid residues.

In conclusion, this study provides valuable insight into Cryo-CMV-GC-MS that can be extended into several other areas of forensics. Future work with the CMV will include fire debris, illicit drug detection, and breath analysis. Additionally, modifying the chemical composition of the sorbent coating will enable sampling of an even wider range

of compounds. Ultimately, the CMV device shows great promise for inexpensive and practical field sampling of volatile analytes with qualitative and quantitative detection.

REFERENCES

[1] Federal Bureau of Investigation, 2015 Crime in the United States: Murder Victims by Weapon, 2016. https://ucr.fbi.gov/crime-in-the-u.s/2015/crime-in-the-u.s.-2015

[2] J.L. Truman, Criminal Victimization, 2015, 2016. http://www.bjs.gov/content/pub/pdf/cv11.pdf.

[3] O. Dalby, D. Butler, J.W. Birkett, Analysis of gunshot residue and associated materials - A review, *J. Forensic Sci*. 55 (2010) 924–943.

[4] J.H. Dillon, The Modified Griess Test: A Chemically Specific Chromophoric Test for Nitrite Compounds in Gunshot Residues, *AFTE J*. 22 (1990).

[5] E. Goudsmits, G.P. Sharples, J.W. Birkett, Recent trends in organic gunshot residue analysis, *Trends Anal. Chem*. 74 (2015) 46–57.

[6] ASTM, Standard Guide for Gunshot Residue Analysis by Scanning Electron Microscopy / Energy Dispersive X-Ray Spectrometry, 95 (2010) 1–5.

[7] A. Martiny, A.P.C. Campos, M.S. Sader, M.A.L. Pinto, SEM/EDS analysis and characterization of gunshot residues from Brazilian lead-free ammunition, *Forensic Sci. Int*. 177 (2008).

[8] W. Fan, J.R. Almirall, High-efficiency headspace sampling of volatile organic compounds in explosives using capillary microextraction of volatiles (CMV) coupled to gas chromatography-mass spectrometry (GC-MS)., *Anal. Bioanal. Chem*. 406 (2014) 2189–95.

[9] A. Tarifa, J.R. Almirall, Fast detection and characterization of organic and inorganic gunshot residues on the hands of suspects by CMV-GC-MS and LIBS, *Sci. Justice*. 55 (2015) 168–175.

[10] A. Tarifa, N. Krietals, J. Mulloor, S. Gura, J.R. Almirall, Cryofocusing Capillary Microextraction of Volatiles (Cryo-CMV) as a novel headspace extraction device for the analysis of volatile organic compounds and smokeless powders, *Forensic Chem*. 3 (2017) 81–89.

[11] Organization of Scientific Area Committees, Comprehensive Feasibility of Organic Gunshot Residue Analysis, 2016. https://www.nist.gov/topics/forensic-science/osac-research-development-needs

[12] H. Meng, B. Caddy, Gunshot Residue Analysis-A Review, *J. Forensic Sci.* 42 (1997) 553–570.

[13] F. Romolo, P. Margot, Identification of gunshot residue: A critical review, *Forensic Sci. Int*. 119 (2001) 195–211.

[14] J.H. Dillon, The Sodium Rhodizonate Test: A Chemically Specific Chromophoric Test for Lead in Gunshot Residues, *AFTE J*. 22 (1990) 251–256.

[15] J.S. Wallace, Chemical Analysis of Firearms, Ammunition, and Gunshot Residue, CRC Press, 2008.

[16] T. Warlow, International Forensic Science and Investigation: Firearms, the Law, and Forensic Ballistics, 3rd ed., CRC Press, Baton Rouge, 2011.

[17] R.V. Taudte, C. Roux, L. Blanes, M. Horder, K.P. Kirkbride, A. Beavis, The development and comparison of collection techniques for inorganic and organic gunshot residues, *Anal. Bioanal. Chem*. 408 (2016) 2567–2576.

[18] M. Wolten, D. Ph, S. Nesbitt, R. Calloway, L. Loper, P.F. Jones, Particle Analysis for the Detection Residue. I : Scanning Electron Microscopy / Energy Dispersive X-Ray Characterization of Hand Deposits, *J. Forensic Sci.* 24 (1979) 409–422.

[19] R.S. Nesbitt, J.E. Wessel, P.F. Jones, Detection of gunshot residue by use of the scanning electron microscope., *J. Forensic Sci.* 21 (1976) 595–610.

[20] G.M. Wolten, R.S. Nesbitt, R. Galloway, G.L. Loper, Particle Analysis for the Detection of Gunshot Gunshot Residue. II : Occupational and and Environmental Particles, *J. Forensic Sci*. 24 (1979) 423–430.

[21] S. Basu, Formation of gunshot residues, *J. Forensic Sci*. 27 (1982) 72–91.

[22] T.R. Kelly, P.H. Bloom, S.G. Torres, Y.Z. Hernandez, R.H. Poppenga, W.M. Boyce, C.K. Johnson, Impact of the california lead ammunition ban on reducing lead exposure in golden eagles and turkey vultures, *PLoS One*. 6 (2011).

[23] M. Demmeler, D. Nowak, R. Schierl, High blood lead levels in recreational indoor-shooters, *Int. Arch. Occup. Environ. Health*. 82 (2009) 539–542.

[24] R.C. Fachehoun, B. Lévesque, P. Dumas, A. St-Louis, M. Dubé, P. Ayotte, Lead exposure through consumption of big game meat in Quebec, Canada: risk assessment and perception, *Food Addit. Contam. Part A. Chem. Anal. Control. Expo. Risk Assess*. 32 (2015) 1501–11.

[25] V.G. Thomas, R. Guitart, Limitations of European Union policy and law for regulating use of lead shot and sinkers: Comparisons with North American regulation, *Environ. Policy Gov.* 20 (2010) 57–72.

[26] V.G. Thomas, Lead-free hunting rifle ammunition: Product availability, price, effectiveness, and role in global wildlife conservation, *Ambio*. 42 (2013) 737–745.

[27] Z. Oommen, S.M. Pierce, Lead-free primer residues: A qualitative characterization of Winchester WinClean™, Remington/UMC LeadLess™, Federal BallistiClean™, and Speer Lawman CleanFire™ handgun ammunition, *J. Forensic Sci*. 51 (2006) 509–519.

[28] R.V. Taudte, A. Beavis, L. Blanes, N. Cole, P. Doble, C. Roux, Detection of gunshot residues using mass spectrometry, *Biomed Res. Int*. (2014).

[29] M.H. Mach, F. Jones, A. Pallos, Feasibility of Gunshot Gunshot Residue Organic Constituents. Part I: Analysis of Smokeless Powders by Combined Gas Chromatography- Chemical Ionization Mass Spectrometry, *J. Forensic Sci*. 23 (1978) 433–445.

[30] E. Goudsmits, G.P. Sharples, J.W. Birkett, Preliminary classification of characteristic organic gunshot residue compounds, *Sci. Justice*. (2016).

[31] M. Maitre, K.P. Kirkbride, M. Horder, C. Roux, A. Beavis, Current perspectives in the interpretation of gunshot residues in forensic science: A review, *Forensic Sci. Int*. 270 (2017) 1–11.

[32] D.M. Northrop, Gunshot Residue Analysis by Micellar Electrokinetic Capillary Electrophoresis: Assessment for Application to Casework. Part 2, *J. Forensic Sci*. (2001) 549–559.

[33] W.A. MacCrehan, M.J. Layman, J.D. Secl, Hair combing to collect organic gunshot residues (OGSR), *Forensic Sci. Int*. 135 (2003) 167–173.

[34] R.Q. Thompson, D.D. Fetterolf, M.L. Miller, R. Mothershead(II), Aqueous Recovery from Cotton Swabs of Organic Explosives Residue Followed by Solid Phase Extraction, *J. Forensic Sci*. 44 (1999) 795–804.

[35] J.D. Twibell, J.M. Home, K.W. Smalldon, D.G. Higgs, T.S. Hayes, Assessment of solvents for the recovery of nitroglycerine from hands using cotton swabs., *J Forensic Sci*. 27 (1982) 792–800.

[36] E. Bernal Morales, A.L. Revilla Vázquez, Simultaneous determination of inorganic and organic gunshot residues by capillary electrophoresis, *J. Chromatogr. A*. 1061 (2004) 225–233.

[37] G. Vanini, R.M. Souza, C.A. Destefani, B.B. Merlo, T.M. Piorotti, E.V.R. de Castro, M.T.W.D. Carneiro, W. Romão, Analysis of gunshot residues produced by .38 caliber handguns using inductively coupled plasma-optical emission spectroscopy (ICP OES), *Microchem. J*. 115 (2014) 106–112.

[38] J.W. Moran, S. Bell, Skin permeation of organic gunshot residue: Implications for sampling and analysis, *Anal. Chem*. 86 (2014) 6071–6079.

[39] J.W. Kilty, Activity after shooting and its effect on the retention of primer residue., *J. Forensic Sci*. 20 (1975) 219–30.

[40] A.M. O'Mahony, J. Wang, Electrochemical detection of gunshot residue for forensic analysis: A review, *Electroanalysis*. 25 (2013) 1341–1358.

[41] T. Jalanti, P. Henchoz, A. Gallusser, M. Bonfanti, The persistence of gunshot residue on shooters' hands, *Sci. Justice*. 39 (1999) 48–52.

[42] J.M.F. Douse, R.N. Smith, Trace analysis of explosives and firearm discharge residues in the metropolitan police forensic science laboratory, *J. Energ. Mater*. 4 (1986) 169–186.

[43] J.B.F. Lloyd, Liquid chromatography of firearms propellants traces, *J. Energ. Mater*. 4 (1986) 239–271.

[44] A. Zeichner, B. Eldar, B. Glattstein, A. Koffman, T. Tamiri, D. Muller, Vacuum collection of gunpowder residues from clothing worn by shooting suspects, and their analysis by GC/TEA, IMS, and GC/MS., *J. Forensic Sci*. 48 (2003) 961–72.

[45] A. Zeichner, B. Eldar, A novel method for extraction and analysis of gunpowder residues on double-side adhesive coated stubs, *J. Forensic Sci*. 49 (2004) 1194–206.

[46] M. Joshi, K. Rigsby, J.R. Almirall, Analysis of the headspace composition of smokeless powders using GC-MS, GC-uECD and ion mobility spectrometry, *Forensic Sci. Int.* 208 (2011) 29–36.

[47] O. Dalby, J.W. Birkett, The evaluation of solid phase micro-extraction fibre types for the analysis of organic components in unburned propellant powders, *J. Chromatogr. A*. 1217 (2010) 7183–7188.

[48] J.L. Thomas, D. Lincoln, B.R. McCord, Separation and Detection of Smokeless Powder Additives by Ultra Performance Liquid Chromatography with Tandem Mass Spectrometry (UPLC/MS/MS), *J. Forensic Sci*. 58 (2013) 609–615.

[49] M. Joshi, Y. Delgado, P. Guerra, H. Lai, J.R. Almirall, Detection of odor signatures of smokeless powders using solid phase microextraction coupled to an ion mobility spectrometer, *Forensic Sci. Int.* 188 (2009) 112–118.

[50] K.H. Chang, C.H. Yew, A.F.L. Abdullah, Optimization of headspace solid-phase microextraction technique for extraction of volatile smokeless powder compounds in forensic applications, *J. Forensic Sci*. 59 (2014) 1100–1108.

[51] M.E. Walsh, Determination of nitroaromatic, nitramine, and nitrate ester explosives in soil by gas chromatography and an electron capture detector, *Talanta*. 54 (2001) 427–438.

[52] ASTM, D1356-15b Standard Terminology Relating to Sampling and Analysis of Atmospheres, 14 (2000) 99–100.

[53] Z.E. Penton, Headspace Gas Chromatography, in: J. Pawliszyn, H. Lord (Eds.), Handb. Sample Prep., Hoboken, NJ, 2010: pp. 25–37.

[54] B. Kolb, L.S. Ettre, Static Headspace-Gas Chromatography: Theory and Practice, Wiley, 2006..

[55] K. Grob, Split and Splitless Injection for Quantitative Gas Chromatography, 4th ed., Wiley, 2001.

[56] H.-J. Hubschmann, Handbook of GC/MS, 1st ed., Wiley, 2001.

[57] M. McMaster, The Mass Spectrometer, in: GC/MS A Pract. User's Guid., 2nd ed., John Wiley & Sons, 2008: pp. 22–66.

[58] M. McMaster, Mass Spectrometer Setup, in: GC/MS A Pract. User's Guid., 2nd ed., John Wiley & Sons, 2008: pp. 67–76.

[59] C. Weyermann, V. Belaud, F. Riva, F.S. Romolo, Analysis of organic volatile residues in 9 mm spent cartridges, *Forensic Sci. Int.* 186 (2009) 29–35.

[60] B. Stevens, S. Bell, K. Adams, Initial evaluation of inlet thermal desorption GC–MS analysis for organic gunshot residue collected from the hands of known shooters, *Forensic Chem.* 2 (2016) 55–62.

[61] O.D. Sparkman, Z. Penton, F.G. Kitson, Gas Chromatography and Mass Spectrometry: A Practical Guide : A Practical Guide (2), Academic Press, Saint Louis, US, 2011.

[62] P. Guerra, H. Lai, J.R. Almirall, Analysis of the volatile chemical markers of explosives using novel solid phase microextraction coupled to ion mobility spectrometry, *J. Sep. Sci.* 31 (2008) 2891–2898.

[63] A. Tarifa, Fast Detection and Chemical Characterization of Gunshot Residues by CMV-GC-MS and LIBS, Florida International University, 2015. http://digitalcommons.fiu.edu/etd/2264

[64] N. Wiebelhaus, D. Hamblin, N.M. Kreitals, J.R. Almirall, Differentiation of marijuana headspace volatiles from other plants and hemp products using capillary microextraction of volatiles (CMV) coupled to gas-chromatography–mass spectrometry (GC–MS), *Forensic Chem.* 2 (2016) 1–8.

[65] D.N.D. Hamblin, Exhaled Breath Analysis of Smokers Using CMV- GC / MS, Florida International University, 2016. http://digitalcommons.fiu.edu/etd/2605

[66] H. Lee, Thermal Design : Heat Sinks, Thermoelectrics, Heat Pipes, Compact Heat Exchangers, and Solar Cells, Wiley, 2010.

[67] A. Tarifa, J. Almirall, Cryofocused Sampling of Volatiles From Air Using Peltier-Assisted Capillary Microextraction, US 15/246,866, 2016.

[68] A.L. Gassner, C. Ribeiro, J. Kobylinska, A. Zeichner, C. Weyermann, Organic gunshot residues: Observations about sampling and transfer mechanisms, *Forensic Sci. Int*. 266 (2016) 369–378.

[69] W. Fan, Improved Dynamic Headspace Sampling and Detection using Capillary Microextraction of Volatiles Coupled to Gas Chromatography Mass Spectrometry, Florida International University, 2013. http://digitalcommons.fiu.edu/etd/982.

[70] A. Beveridge, Forensic Investigation of Explosions, CRC Press, 1998.

[71] M. Harper, Sorbent trapping of volatile organic compounds from air, *J. Chromatogr. A*. 885 (2000) 129–151.

[72] E. Woolfenden, Sorbent-based sampling methods for volatile and semi-volatile organic compounds in air. Part 2. Sorbent selection and other aspects of optimizing air monitoring methods, *J. Chromatogr. A*. 1217 (2010) 2685–2694.