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Fast Detection and Chemical Characterization of Gunshot Residues by CMV-GC-MS and LIBS

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FAST DETECTION AND CHEMICAL CHARACTERIZATION OF GUNSHOT RESIDUES BY CMV-GC-MS AND LIBS

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by

Anamary Tarifa

2015

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

This dissertation, written by Anamary Tarifa, and entitled Fast Detection and Chemical Characterization of Gunshot Residues by CMV-GC-MS and LIBS, having been approved in respect to style and intellectual content, is referred to you for judgment.

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

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DEDICATION

This dissertation is dedicated to God and my family: to God because in Him all things are possible, to my mother and father because your sacrifices have enabled me to pursue a higher education degree and to my brother because your love and life have inspired me. I also dedicate this work to my husband; I would not have been able to complete this work without your support, encouragement and unconditional love.

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ABSTRACT OF THE DISSERTATION

FAST DETECTION AND CHEMICAL CHARACTERIZATION OF GUNSHOT RESIDUES BY CMV-GC-MS AND LIBS

by

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Gunshot residue (GSR) is the term used to describe the particles originating from different parts of the firearm and ammunition during the discharge. A fast and practical field tool to detect the presence of GSR can assist law enforcement in the accurate identification of subjects.

A novel field sampling device is presented for the first time for the fast detection and quantitation of volatile organic compounds (VOCs). The capillary microextraction of volatiles (CMV) is a headspace sampling technique that provides fast results (< 2 min. sampling time) and is reported as a versatile and high-efficiency sampling tool. The CMV device can be coupled to a Gas Chromatography-Mass Spectrometry (GC-MS) instrument by installation of a thermal separation probe in the injection port of the GC.

An analytical method using the CMV device was developed for the detection of 17 compounds commonly found in polluted environments. The acceptability of the CMV as a field sampling method for the detection of VOCs is demonstrated by following the criteria established by the Environmental Protection Agency (EPA) compendium method TO-17.

The CMV device was used, for the first time, for the detection of VOCs on swabs from the hands of shooters, and non-shooters and spent cartridges from different types of ammunition (i.e., pistol, rifle, and shotgun). The proposed method consists in the headspace extraction of VOCs in smokeless powders present in the propellant of ammunition. The sensitivity of this method was demonstrated with method detection limits (MDLs) 4-26 ng for diphenylamine (DPA), nitroglycerine (NG), 2,4-dinitrotoluene (2,4-DNT), and ethyl centralite (EC).

In addition, a fast method was developed for the detection of the inorganic components (i.e., Ba, Pb, and Sb) characteristic of GSR presence by Laser Induced Breakdown Spectroscopy (LIBS). Advantages of LIBS include fast analysis (~ 12 seconds per sample) and good sensitivity, with expected MDLs in the range of 0.1-20 ng for target elements.

Statistical analysis of the results using both techniques was performed to determine any correlation between the variables analyzed. This work demonstrates that the information collected from the analysis of organic components has the potential to improve the detection of GSR.

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LIST OF ABBREVIATIONS AND ACRONYMS

2,4-DNT	2,4-Dinitrotoluene
ASTM	American Society for Testing and Materials
CCD	Charge-coupled device
CMV	Capillary microextraction of volatiles
DPA	Diphenylamine
EC	Ethyl centralite
ECD	Electron capture detector
EPA	US Environmental Protection Agency
EIC	Extract ion chromatograms
FOC	Fiber optic cable
GC	Gas chromatography
iCCD	Intensified charge-coupled device
IP	Ionization potential
IR	Infrared
ICP-OES	Inductively coupled plasma optical emission spectrometry
LDA	Linear discriminant analysis
LDF	Linear discriminant function
LDR	Linear dynamic range
LIBS	Laser-induced breakdown spectroscopy
LOD	Limit of detection
LOQ	Limit of quantitation
MDL	Method detection limit

MQL	Method quantitation limit
MS	Mass spectrometry
NAA	Neutron activation analysis
Nd:YAG	Neodymium-doped yttrium aluminum garnet
NG	Nitroglycerine
NIST	National Institute of Standards and Technology
PCA	Principal components analysis
PDMS	Polydimethylsiloxane
PLSDA	Partial least square discriminant analysis
PSPME	Planar solid phase microextraction
QC	Quality control
RF	Radio frequency
RSD	Relative standard deviation
SA	Self-absorption
SCD	Segmented-array charge-could device
SEM-EDS	Scanning electron microscopy – energy dispersive spectroscopy
SNR	Signal-to-noise ratio
SPME	Solid phase microextraction
XRF	X-ray fluorescence

1 INTRODUCTION

1.1 Research motivation

The portability of analytical instrumentation for field analysis is an attractive choice for law enforcement and environmental agencies. Portable instruments and tools have been developed with several applications in illicit drugs and explosives detection, environmental monitoring, and food authentication [Perr et al., 2005; Guerra et al., 2008; Wong et al., 2013; Huang et al., 2015; Soria et al., 2015]. Some of the instruments that have been adapted for field analysis include: Gas chromatography mass spectrometry (GC-MS), ion mobility spectrometry (IMS), x-ray fluorescence spectroscopy (XRF), and laser induced breakdown spectroscopy (LIBS) [Fortes et al., 2010]. In addition, commonly used field sampling techniques include: solid phase microextraction (SPME), purge and trap, and sorbent tubes [Joshi et al., 2009, Wong et al., 2013; Soria et al., 2015].

The first portable LIBS system was developed by Cremers et al., at the Los Alamos National Laboratory. The instrument was successfully used for the detection of lead (Pb) and other metals in paint and soil [Rakovský et al., 2014]. In addition, the use of LIBS has been employed in the detection of explosives in fingerprints. It was reported that the sensor system was 31 m from the target, and the laser consisted of a double pulse Nd:YAG laser system. The detection of dinitrotoluene (DNT), trinitrotoluene (TNT), research department formula X (RDX), and pentaerythritol tetranitrate (PETN) was possible with this system by looking at the CN emission lines [Lucena et al., 2013].

The detection of drugs and explosives has also been performed by SPME-IMS. Perr et al., (2005) published the first peer reviewed report for the coupling of SPME with

a bench top IMS system. The detection of compounds present in smokeless powder was successful with detection limits in the range of 0.16-0.57 ng. These studies were performed by spiking an amount of the standard compound in a quart can at room temperature [Perr et al., 2005]. The detection of diphenylamine (DPA), ethyl centralite (EC), 2-ethyl 1-hexanol, and 2,4-dinitrotoluene (2,4-DNT) was reported in smokeless powders where DPA was found in all the samples (n=5), while EC and 2,4-DNT were found in 2 and 3 of the samples [Joshi et al., 2009]. In a similar study, SPME extraction capability was compared to planar solid phase microextraction for the analysis of explosives in IMS. The results yield greater amount of TNT being extracted by PSPME [Guerra et al., 2008].

In the present work, a novel headspace extraction technique, Capillary Microextraction of Volatiles (CMV) will be evaluated, for the first time, for the fast detection of volatile organic compounds in ambient air to determine the presence of gunshot residues (GSR). The potential applicability of CMV as a field sampling device will be demonstrated with the headspace analysis of indoor air samples and GSR samples from the hands of shooters.

The detection of volatile organic compounds (VOCs) present in ambient air is of great concern because of the potential hazards to human health and the environment [Dou et al., 2011; Wong et al., 2013]. The Environmental Protection Agency (EPA) has created an extensive list of compounds that have been reportedly detected in areas where air pollution is suspected such as, industrial sites [EPA TO-17]. Therefore, there is a need for the detection, monitoring, and quantitation of VOCs in ambient air. In an effort to address this issue, the EPA has published the “Compendium of Methods for Toxic Organic Air

Pollutants" since 1984 (TO-1 to TO-17). These are a series of reports describing the most current methods and guidelines to be followed for the monitoring of VOCs in ambient air and polluted environments.

Additionally, the use of firearms has become prominent in multiple terrorist attacks, school massacres, and police-hatred attacks. In these cases, the forensic evidence collected includes: the firearm, spent cartridges, bullets, ammunition, and gunshot residues. The spent cartridges and bullets contain unique markings created by the mechanical operation of the weapon. Thus, the evidence can provide information on whether a particular spent cartridge or bullet was fired with the suspected weapon and ultimately link the weapon to a suspect. Gunshot residues (GSR) can also provide valuable evidence in searching for a suspect. However, current techniques are presumptive in nature (e.g., color tests) or may take several hours before the sample is analyzed (e.g., elemental analysis). Consequently, law enforcement agencies need fast and practical tools for the analysis of forensic evidence, in firearm related crimes.

This dissertation presents a practical approach to gunshot residue analysis, to provide a fast and reliable tool to law enforcement for the unambiguous detection of gunshot residue. The headspace extraction of organic compounds in GSR was performed with CMV devices. In addition, elemental analysis of GSR will be performed by Laser Induced Breakdown Spectroscopy (LIBS). The ultimate goal of this work is to apply statistical analysis tool that will correctly associate shooters from the detection of GSR collected from their hands.

The following paragraphs provide a summary of the information that can be obtained from GSR analysis and some of the techniques used for analysis. Other sections

in the following chapters will provide a more detailed discussion of gunshot residue collection and analysis.

When a firearm is discharged, partially burnt and unburned propellant powder, as well as primer components and combustion materials, escape from the weapon and are deposited around the area of discharge [Dalby et al., 2010]. The combinations of inorganic and organic components created as a result of firearm discharge are known as gunshot residues (GSR). Inorganic component particles originate from the primer cup and mixture, cartridge case, propellant powder, bullet, projectile jacket, and the barrel of the weapon [Dalby et al., 2010]. The organic components mainly originate from the smokeless powders used in the manufacture of explosives and are the main components of propellants in firearm ammunition. Smokeless powders in propellant are classified as low explosives because discharge occurs in a closed system created by the casing, which holds together all the components of the ammunition [Midkiff et al., 2002]. Other organic materials are also generated from the primer mixture and firearm lubricants [Dalby et al., 2010].

Most of the firearms cases that are analyzed in the lab require the identification of a suspect that may have been involved in the crime and who could be linked to the weapon with which the crime was committed. Many forensic laboratories focus on the comparison of spent cartridges collected from the crime scene by studying physical characteristics such as markings from the manufacturing process and the firing pin of the weapon. Also, the markings on the bullet are created during discharge from lands and grooves made to the barrel of the gun during the manufacturing process and can indicate which firearm was used in the crime through comparison tests. These studies are mainly

performed by physical comparison analysis using comparison microscopes [Midkiff et al., 2002].

An alternative method to link a suspect to a crime involving firearm discharge is through GSR analysis. The analysis of GSR examines the presence of particles with an inorganic composition of barium (Ba), lead (Pb), and antimony (Sb). The method of choice in forensic laboratories for the analysis of GSR is Scanning Electron Microscopy (SEM) coupled to a Wavelength Dispersion X-ray Spectroscopy (WDS) or an Energy Dispersion X-ray Spectroscopy (EDS) detector to obtain both morphological and elemental information from the particles [Dalby et al., 2010; Brożec-Mucha et al., 2011]. Firearm discharge residues also contain particles that are composed of volatile organic compounds because these particles mainly originate from the smokeless powders in the propellant [Dalby et al., 2010].

Other techniques have been applied for the analysis of organic and inorganic components in GSR. For the organic components, extraction of volatile compounds has been performed using Solid Phase Microextraction (SPME) and analyzed by Gas Chromatography (GC) coupled to different detectors such as Flame Ionization (FID), Thermal Energy Analyzer (TEA), Electron Capture (ECD), and Mass Spectrometry (MS). In addition, solvent extraction followed by High Pressure Liquid Chromatograghy-Mass Spectrometry (HPLC-MS) has been applied as well as Capillary Electrophoresis (EC) and Ion Mobility Spectrometry (IMS) [Dalby et al., 2010; Brożec-Mucha et al., 2011; Arndt et al. 2012]. The techniques used for the analysis of inorganic components include: Atomic Absorption Spectrometry (AAS), Neutron Activation Analysis (NAA), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), Inductively Coupled Plasma-

Atomic Emission Spectroscopy (ICP-AES), Raman Spectroscopy, X-ray Fluorescence (XRF), and Laser Induced Breakdown Spectroscopy (LIBS) [Dockery et al., 2003; Brożec-Mucha et al., 2009; Dalby et al., 2010; Michel et al., 2010; Kumar et al., 2011; Charles et al., 2011; Brożec-Mucha et al., 2011].

The biggest limitation of most of these techniques is the extensive analysis time. For instance, the amount of time required to find GSR particles mounted on an aluminum stub with carbon adhesive by SEM-EDS ranges from 6-8 hours. Other techniques are selective, but may exclude an element of interest such as Pb, as in the case of analysis performed by NAA. Also an x-ray detection technique such as XRF is not sensitive enough to analyze a particle that is micrometers in size (<10 μm) because of its large beam area (100 μm) [Flynn et al., 1998].

In the current study, an innovative headspace extraction technique is utilized for the first time in the detection of volatile organic compounds characteristic of GSR and air samples contaminated with BTEX (Benzene, Toluene, Ethylbenzene, and Xylenes) compounds. The CMV device is a novel extraction method previously reported, demonstrating improved sensitivity and selectivity comparable to SPME, for the extraction of volatiles in the headspace of smokeless powders [Fan et al., 2013].

Also, one of the objectives of this work is to develop a method for the unambiguous identification of GSR by combining the results obtained from inorganic and organic components. Laser Induced Breakdown Spectroscopy (LIBS) is the technique of choice for the analysis of inorganic components in GSR because of the following capabilities: fast analysis time, simultaneous multi-elemental detection, portability for field analysis, and the ability to provide quantitative results.

1.2 Significance of the study

The significance of this research include: the practical application of a novel headspace sampling technique (CMV-GC-MS) for the analysis of volatile organic compounds in GSR and indoor air, and method development of a fast technique (LIBS) for inorganic components analysis of GSR. In addition, data fusion of the organic and inorganic components in GSR will provide a statistical tool to calculate the correct association rates.

One of the goals of this project is to demonstrate the capabilities of CMV over commercially available sampling techniques (i.e. sorbent tubes). The performance of CMV devices was compared to commercially available sorbent tubes, which are commonly used in the analysis of ambient air. The applicability of CMV for analysis of ambient air was demonstrated by following the criteria established by the EPA Compendium Method TO-17. A brief introduction and full discussion of results will be presented in Chapter 2 of this dissertation.

In addition, a CMV-GC-MS method was developed and optimized for the detection of VOCs on the swabs collected from the hands of individuals. A total of 43 police officers and 20 individuals in a control group participated in this study. The hands of each person were swabbed and the samples were transported to the lab for analysis. Additionally, headspace extraction over 6 cotton swabs was performed to identify the background profile from blank swabs.

For inorganic components analysis, a LIBS method was developed and optimized for the detection of elements indicative of GSR presence on the hands of a shooter. The efficiency of LIBS to detect the target elements was confirmed by ICP-OES because it is

also a spectroscopy technique and will provide a similar output. The elemental composition of cotton from blank swabs was evaluated by analyzing 10 swabs by LIBS and 20 swabs by ICP-OES. The same samples analyzed by CMV-GC-MS were also treated and analyzed by LIBS. Confirmation of the elemental profile was performed for all samples by solution ICP-OES.

The analysis of GSR collected from the hands of police officers allowed the evaluation of the performance for the different analytical techniques as well as the determination of correct association rates, demonstrating the suitability of LIBS for the elemental analysis of GSR. Ultimately, this work demonstrates for the first time the utility of CMV devices for the headspace extraction of VOC's indicative of GSR presence.

This dissertation also presents the development of a practical statistical approach by combining the information obtained from the presence of both the organic and inorganic components in GSR. The results obtained in this work will demonstrate the capabilities of the developed methods for the analysis of GSR in the field.

The instrumentation used in this work, LIBS and GC-MS, have been previously developed into portable systems for field sampling and are commercially available [Bednar et al., 2012; Liaud et al., 2014; Rakovský et al., 2014]. The results obtained through this research will aid law enforcement and environmental agencies in the detection of GSR and VOCs in contaminated air with faster analysis time and with the use of commercially available portable systems.

2 EVALUATION OF A NOVEL CMV DEVICE FOR THE ANALYSIS OF VOLATILE ORGANIC COMPOUNDS

2.1 Analysis of volatile organic compounds in ambient air

The detection of volatile organic compounds (VOCs) present in ambient air is of great concern because of the potential hazards to human health and the environment [Wong et al., 2013; Dou et al., 2011]. The Environmental Protection Agency (EPA) has created an extensive list of compounds that have been reportedly detected in areas where air pollution is suspected, such as industrial sites [EPA TO-17]. Many of the compounds present in ambient air have the potential to act as mutagens and carcinogens [Wong et al., 2013; Dou et al., 2011]. Therefore, there is the need for the detection, monitoring, and quantitation of VOCs in ambient air. In an effort to address this issue, the EPA has published the "Compendium of Methods for Toxic Organic Air Pollutants" since 1984 (TO-1 to TO-17). These are a series of reports describing the most current methods and guidelines to be followed for the monitoring of VOCs in ambient air or polluted environments.

2.1.1 Analysis of VOCs with sorbent tubes

The analysis of VOCs in ambient air is currently performed with sorbent tubes following the guidelines from the EPA method TO-17. The commercially available sorbent tubes consist of a thin cylinder that can be made out of glass or stainless steel. A physical portion of the tubes are packed with sorbent material, thus the name sorbent tubes.

Commonly used sorbent materials include: several variations of Tenax®, Carbotrap®, and Carbopack®, as well as a combination of materials in the same tube [Gallego et al., 2010]. The sorbent material of choice depends heavily on the targeted compounds, specifically the volatility or vapor pressure of the molecule. In addition, the sorbent material can be classified according to its strength, which is described as the sorbent affinity to most of the VOC analytes. The sorbent strength is related to the surface area of the sorbent material. A weak sorbent has a surface area less than 50 m^2/g, a medium sorbent has a surface area in the range of 100-500 m^2/g, and a strong sorbent has a surface area around 1000 m^2/g [EPA TO-17]. In general, stronger sorbents are used for highly volatile compounds.

Some of the limitations observed for analysis of VOCs with sorbent tubes include: long headspace extraction times (~1 hr) with low flow rate, and the use of complex and expensive thermal desorption units for analysis with a GC-MS [Oliver et al., 1996; Daughtrey et al., 2001].

2.1.2 Evaluation criteria for the analysis of VOCs by CMV

The applicability of CMV devices for the detection of VOCs in ambient air will be demonstrated by complying with the guidelines of the EPA method TO-17. There are four performance criteria that should be met to qualify under Compendium Method TO-17: 1) method detection limit of 0.5 ppbv or less, 2) analytical precision of 20%, 3) precision for distributed volume pair of 25% or less, and 4) an audit accuracy within 30% for concentrations expected in contaminated ambient air (0.5 to 25 ppbv).

The method detection limit (MDL) is calculated by obtaining 10 replicate blank samples and using the following equation:

$$MDL = 3 \times \sigma_{standard\ deviation\ of\ the\ noise} \quad \text{Equation 1}$$

Then the MDL is confirmed by obtaining seven replicate measurements of a concentration close to the expected detection limit, as specified in method TO-17. Finally, the standard deviation of the seven measurements is multiplied by 3.14 (Student's t value for 99% confidence) to obtain the limit of detection.

The analytical precision was calculated with the following equation:

$$Analytical\ precision = \frac{|X_1 - X_2|}{\bar{X}} \times 100 \quad \text{Equation 2}$$

were X_1 is the measurement value performed with one sorbent tube, X_2 is the measurement value performed with a second sorbent tube, and $\bar{X}$ is the average of the two measurements (i.e. X_1 and X_2). There are a number of factors that may hinder precision, such as artifact formation and breakthrough of target compounds.

The distributed volume pairs are used for the extraction of unknown content in ambient air (e.g., 1 L and 4 L sampling volumes). The precision of distributed volume pair is calculated as a percentage of the relative difference between distributed volume pair as follows:

$$Percent\ difference = \frac{X_1 - X_2}{\bar{X}} \times 100 \quad \text{Equation 3}$$

were X_1 is one measurement value (e.g., 1 L sampling volume), X_2 is a second measurement value (e.g., 4 L sampling volume), and $\bar{X}$ is the average of the two measurements (i.e. X_1 and X_2). Ideally the amount detected for each compound should have a linear correlation with respect to the sampling volume.

The forth criteria is the audit accuracy, which refers to how much the detected amount of analyte differs from the nominal amount. The audit accuracy can be calculated using the following equation:

$$\%\ Audit\ accuracy = \frac{Spiked\ amount - Detected\ amount}{Spiked\ amount} \times 100 \qquad \text{Equation 4}$$

All the equations used were obtained from the EPA Compendium Method TO-17.

In addition to these 4 criteria, the EPA TO-17 mentions that the breakthrough of the sorbent tubes should be less than 5%. The breakthrough is defined as the amount of VOCs detected at the end of the sampling sorbent tube [EPA TO-17]. Breakthrough is measured by connecting two sorbent tubes in series and calculating the percentage of VOCs present in the back sorbent tube with respect to the amount collected from both tubes.

2.2 Fundamentals of CMV for headspace extraction

The analysis of volatile organic compounds (VOCs) is of particular importance for environmental agencies in the detection of toxic components in ambient air as well as in the detection of fire debris and explosives. There have been several approaches to the detection and analysis of these compounds by GC-MS including several headspace extraction techniques. The most commonly known headspace extraction techniques are: purge and trap, solid phase microextraction (SPME), and sorbent tubes.

The capillary microextractor of volatiles (CMV) device is a novel extraction method previously reported, demonstrating improved sensitivity and selectivity comparable to SPME, for the extraction of volatiles in the headspace of smokeless powders [Fan et al., 2013].

2.2.1 Principles and capabilities of CMV

The CMV consists of an open ended glass capillary packed with sorbent coated glass filters (i.e., PSPME). The inner diameter of the glass capillary is 2 mm and cut into 2 cm long. The PSPME is a glass filter coated with vinyl terminated polydimethylsiloxane (PDMS). The PSPME is cut into rectangular pieces measuring 2 cm by 2 mm and are used to pack the glass capillaries. Approximately 7 pieces of coated glass filters can be packed inside the 2 cm glass capillary. Figure 1a shows a photograph of a CMV device once it is packed with the PSPME.

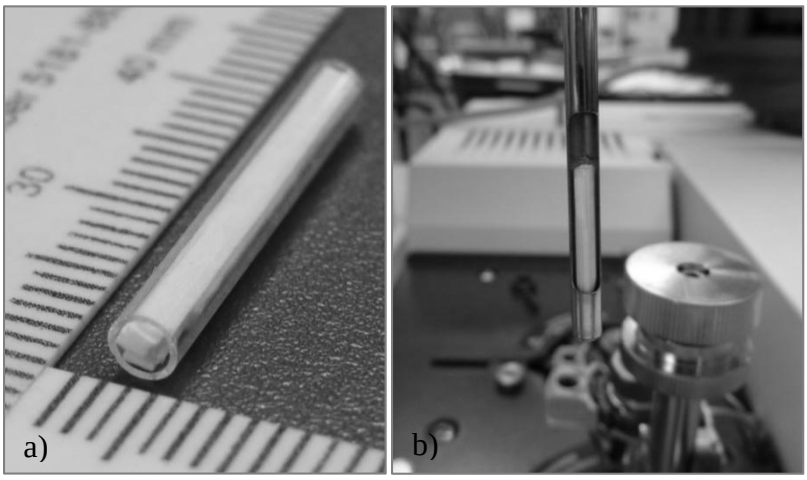

Figure 1. a) The CMV device with dimensions of 2 cm long and an inner diameter of 2 mm and b) the device in the thermal separation probe (TSP) for introduction into the GC inlet

As shown in Figure 1, the CMV has both ends open, which can be connected to a pump for headspace extraction. The surface area of CMV is 0.05 m^2 and has a phase volume of 100 mm^3 which is greater than the value for SPME [Fan et al., 2013]. Therefore, it provides more capacity for compounds than SPME ($9.4x10^{-6}$ m^2). In

addition, the PDMS coating on the glass filters is hydrophobic, which improves extraction of VOCs in humid conditions.

The CMV fundamentals can be explained in terms of chromatographic principles. When the CMV is attached to the pump, air flows (mobile phase) through the device and the compounds will undergo partitioning with the PDMS coating (stationary phase). Interactions between the mobile and stationary phase can be defined in terms of the distribution constant (K_D), which is a ratio of the concentration of the compounds in the stationary phase over the mobile phase. Therefore, by expressing this ratio in terms of mass per volume, the amount of a particular compound extracted in the PDMS phase can be calculated.

Another method for calculating K_D is with the retention time (t_R), which in the CMV it represents the movement of the compound from one end of the device to the other. Thus, following chromatographic principles, the concentration of a compound in the device is directly related to extraction time and initial concentration [Robards 2004].

2.2.2 Thermal separation probe

A thermal separation probe is a sample holder that can be introduced directly into the injection port of a GC system. The probe comes with a metal unit that is installed on the injection port of the GC, and the sample can be introduced with the aid of a probe as shown in Figure 1b.

The TSP typically comes with micro vials that are used to introduce liquid or solid samples in the GC. Heating the sample produces vapors from volatile substances that can then be introduced in the analytical column by the carrier gas.

The advantages of the TSP over headspace autosamplers is that all the vapors produced can be introduced in the GC, whereas the autosamplers remove only a small portion of the headspace. In addition, the introduction of CMV into the injection port with TSP requires little to no sample preparation and minimizes loss from the sample in a transfer line as is the case for headspace samplers.

For the purpose of this project, after performing headspace extraction, the VOCs are absorbed to the PDMS coating of the glass filter inside the CMV and thermal desorption can be achieved by introducing the CMV in the thermal separation probe as shown in Figure 1b.

2.3 Fundamentals and principles of gas chromatography

Gas chromatography (GC) is a separation technique mainly for the analysis of organic compounds. The best application for GC technique is the analysis of compounds in a mixture solution as it will provide separation followed by detection of individual compounds. Gas chromatography is the technique of choice for the analysis of many different matrixes such as ambient air, drugs, food, and explosives [Perr et al., 2005; Fan et al., 2013; Soria et al., 2015].

The GC system consists of an injection port, a capillary column, an oven, a detector, and a computer to translate and process the data. In general, a liquid sample is introduced in the injection port, where the sample is vaporized, and carried into the capillary column by a flow of gas, usually He. Once the sample is in the capillary column, the different compounds are separated depending on the affinity of the compounds with the inside coating of the column. Finally, the individual compounds will

elute from the column and reach the detector. The signal detected will be translated by a computer into a chromatograph showing elution time versus intensity for all the individual compounds.

There have been several improvements to GC over the years. One of these improvements is the introduction of liquid or gaseous analytes, from liquid, solid, or gas samples. The most commonly used sample introduction system is the autosampler to inject liquid solutions into the GC. Gaseous samples can also be analyzed by modifying the sample introduction system. The introduction of gaseous samples can be performed using a headspace autosampler and SPME techniques.

Once the sample is introduced into the injection port, the sample is vaporized and all or some of the sample is introduced into the analytical column by a flow of carrier gas. The volume injected depends on the expansion of the gas sample after vaporization inside the injection port. The common injection volume used is 1 µL, but some applications require larger sample volumes [Robards 2004]. For liquid samples, injection is usually performed in split mode to remove most of the solvent and introduce some of the sample into the analytical column. Splitting the sample can be achieved by opening the split valve and allowing a certain flow of gas to vent. The split ratio can be calculated by dividing the split flow over the overall gas flow.

Separation of the individual components in the sample will take place inside the analytical column. Generally, the analytical column consists of a glass capillary column with a diameter in the µm size range and comes in a variety of lengths (5-60 m) depending on the application [Wong et al., 2013; Fan et al., 2013]. In GC, the capillary column is coated inside with a few µm of a solid material which is known as the

stationary phase. The carrier gas transfers the sample towards the end of the column, thus it is called the mobile phase.

Separation of the components in the capillary column depends on the affinity of the compounds to the stationary phase of the column. Overall, compounds having greater affinity with the stationary phase will spend more time in this phase than in the mobile phase [Scott 2003]. Each compound will have different degrees of affinity with the stationary phase, which further enables separation of the compounds. The stationary phase can be made of materials with different chemical characteristics depending on the compounds of interest [Robards 2001].

Another phenomenon occurring in the separation process is the partitioning of the compounds with the stationary phase. Because there is also a mobile phase, the compounds are constantly moving in and out of the stationary phase. The partitioning process continues until the individual compounds elute at the end of the capillary column [Robards 2001].

The elution of compounds is also affected by differences in boiling point temperatures. Overall, compounds with lower boiling point temperatures will elute first, and those with higher boiling point temperature will elute last. To further control the time of elution for compounds, the capillary column is inside a temperature programmable oven. A typical temperature program or ramp program will start with a low temperature and increase the temperature gradually until a set point, at which time all compounds should have eluted [Scott 2003].

Each compound elutes at a specific retention time under the same conditions (i.e., every time the same stationary phase and separation parameters are used). Therefore,

identification by retention time is possible, although with the limitation that there could be several compounds with the same retention time.

In order to improve the identification of compounds by chromatography and because of the versatility of the method, other systems can be coupled to GC. Some of the analytical methods that have been coupled to GC are: Ultraviolet Detector (UV), Flame Ionization Detector (FID), Electron Capture Detector (ECD), and Mass Spectrometry (MS) [Robards 2001].

Once the individual compounds reach the detector, the signal will be translated and processed through a computer. The output of the data is a chromatograph that shows the retention time for each compound versus the signal intensity. Additional information from each compound can be obtained depending on the detection system coupled to the GC.

In the following sections only the techniques of interest (GC-MS and GC-μECD) will be discussed in more detail.

2.3.1 Principles and capabilities of GC-MS

Gas Chromatography Mass Spectrometry (GC-MS) is considered to be the “gold standard” for identification and quantitation of samples. The technique has multiple advantages including the unequivocal identification of compounds by retention time and mass-to-charge ratio profile of the molecule.

Mass spectrometry consists of the separation of compounds by mass-to-charge ratio followed by detection. The different components of a mass analyzer system include:

the transfer line, the ionization source, the mass analyzer, the vacuum pump, the detector, and a computer to translate and process the data.

The transfer line is the component that connects the GC to the MS system. The analytical column from the GC passes through the transfer line which is maintained at a constant temperature, usually 20°C higher than the last ramp temperature. The reason for having the transfer line at a high temperature is to avoid condensation of the sample going from the GC to the MS as there is still an ambient air gap between the GC and the MS.

The analytical column will extend all the way through the transfer line and stop a few millimeters away from the ionization source. The ionization process depends on the type of source used. There are two well-known ionization sources: electron impact (EI) source and chemical ionization (CI) source. The EI source is the most commonly used and is considered a hard ionization source because it produces extensive fragmentation of the molecule. The CI source is considered to be a soft ionization source because it produces less fragmentation of the molecule, thus it provides information about the molecular ion [Hoffmann 2004].

The choice of ionization source depends on the target compounds and the information that wants to be acquired. As mentioned before, the EI source produces more fragmentation of the molecule and information of molecular weight is not always acquired for this reason. On the other hand, the CI source is more prompt to provide information about the molecular weight of the compound. The molecular weight of a compound is particularly important when identifying an unknown compound. In other

cases, information obtained from fragmentation with the EI source can be enough for identification of a compound.

Ionization by EI consists of a stream of electrons created with a tungsten filament that strikes the compounds as these elute from the capillary column. The filament is usually operated at 70eV, only 10eV is enough to ionize the molecule, thus the remainder of the energy will produce extensive fragmentation of the molecules [Hoffmann 2004]. As the name implies, ionization of the molecule occurs by impact of the electrons generated in the filament with the gaseous molecule. The electron ionization of a molecule occurs with the following process:

$$M + e^- \rightarrow M^{\bullet +} + 2e^-$$

were M represent the molecule and $M^{\bullet +}$ is the molecular ion.

Each molecule undergoes characteristic fragmentation into ions, radicals, excited species, and neutral species. The ions fragmentation form a profile for each compound which is used for identification of molecules Figure 2 shows a schematic of an electron impact source [Hoffmann 2004]. Once ionization of the molecules occurs, only ions with a specific m/z ratio can pass through the mass analyzer and be transferred to the detector.

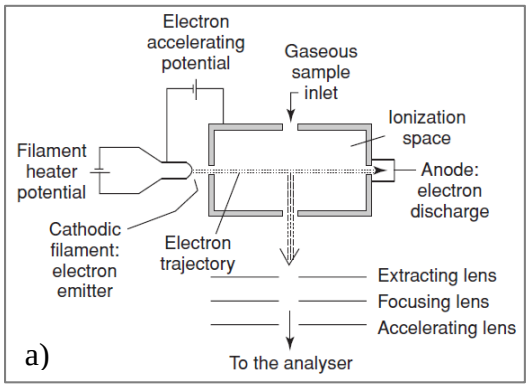

Figure 2. Schematic of an EI source

Mass analyzers can be classified as time-resolved or space-resolved. Time-resolved mass analyzers operate by allowing only selected ions to pass through and reach the detector. Space-resolved mass analyzers confine ions to an area and only ions with a specific mass-to-charge ratio can reach the detector.

The quadrupole mass analyzer is the most commonly used for chromatographic analysis because these are compact units, less expensive, and have lower scan times. The quadrupole consists of four parallel rods with an applied alternating electric field that acts as a mass filter. Figure 3 is a schematic of a quadrupole mass analyzer with all the corresponding parts [Hoffmann 2004]. Before entering the quadrupole the generated ions are pulled into the space where a series of lenses focus the ion beam to be transferred to the mass analyzer.

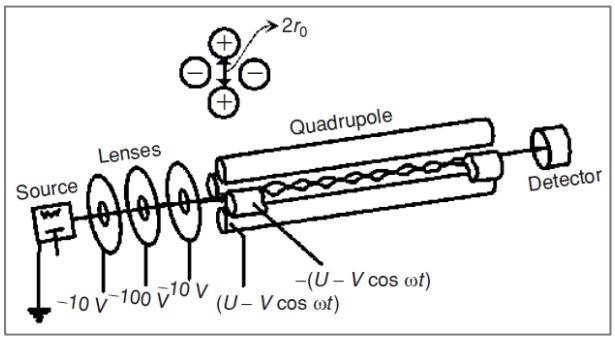

Figure 3. Schematic of a single quadrupole mass analyzer showing the trajectory of ions

Inside the mass analyzers, the ions travel in a free path until these reach the detector. Only the ions with a specific mass to charge ratio can reach the detector. The rest of the ions, neutrals, and excited species will collide with the rods or will be pulled out by the vacuum pump. The vacuum pump is an essential part of the system because it

is responsible for allowing the ions to move in a free path to reach the detector without collisions with other molecules [Hoffmann 2004].

The principle of the quadrupole was described by Paul and Steinwegen in 1953. In order to control the trajectory of the ions, a direct current (DC) and an alternative current (AC) are applied to the rods or electrodes. In this way if a positive ion enters the space between the rods, the ion will be attracted to the negative rod. Thus, alternating the current will make the ions travel in an oscillatory manner as depicted in Figure 3. Only the ions with a stable trajectory and therefore with a specific mass to charge ratio (m/z) will reach the detector.

The typical detector used in GC-MS is the electron multiplier detector (EM). In this detector ions with a specific m/z are first accelerated by an electrode (conversion dynode) at high potential (± 3-30 kV) which is opposite to the charge of the ions. When a positive ion strike the negative high voltage conversion dynode, negative ions and electrons are produced. These secondary particles are converted to electrons at the first dynode and are amplified by a cascade effect in the electron multiplier, which produces a current. The electrical current produce is amplified by conventional electronic amplification, which is then translated to produce a signal [Hoffmann 2004].

2.4 Experimental

2.4.1 Instrumentation

2.4.1.1 Analysis of VOCs by CMV-GC-MS

The analysis of VOCs extracted with the CMV devices was performed with a gas chromatograph coupled to a single quadrupole mass spectrometer (GC-MS) equipped

with a μECD detector. The GC-MS consists of an Agilent Technologies (Santa Clara, CA) GC system 7890A and a GC/MS Single Quad 5975C. The GC system is equipped with a Pneumatics Control Module (PCM), which allows the coupling of the analytical column to both the single quadrupole and the μECD detector.

A Thermal Separation Probe (TSP) (Agilent Technologies, Santa Clara, CA) with a 4mm ID liner was used to thermally desorb the CMV devices into the GC-MS injector.

The analytical column used for the present study was a 29.17 m DB-5ms Ultra Inert with 0.25 mm inner diameter, and a film thickness of 0.25 μm. The GC oven ramp temperature started at 35°C with a 1 min hold at 35°C then the temperature was increased to 120°C at 15°C/min. The temperature was then increased to 220°C at 30°C /min and held for 1.5 min at that temperature. The final temperature reached was 280°C at 30°C /min and held for 1 min. The total time for the chromatographic separation was 14.50 min. The injector temperature was set at 180°C in split (split ratio 5:1) with a column flow of 1.2 mL/min. The EI source was kept at 230°C, the transfer line to the mass spectrometer was set to 280°C and the quadrupoles were maintained at 150°C. The scan mass range was set at 45-300 amu. The resolution of the mass analyzer is 0.1amu. The instrument was tune before each experiment using the autotune feature as recommended by the manufacture.

The analytical performance of several compounds expected to be present in ambient and polluted air environments was evaluated. The targeted compounds consisted of: Dichloromethane (Methylene chloride), benzene, pyridine, toluene, furfural, ethylbenzene, m-xylene, p-xylene, o-xylene, benzaldehyde, phenol, benzonitrile, 1,2,4-trimethylbenzene, 1,2,3-trimethylbenzene, acetophenone, nonanal, and naphthalene. The

retention time and mass spectra profile for each compound was obtained from injecting a standard solution in the GC system.

2.4.2 Reagent and standards

For optimization studies and calibration curves, single compounds standard solutions of dichloromethane, benzene, pyridine, toluene, furfural, ethylbenzene, m-xylene, p-xylene, o-xylene, benzaldehyde, phenol, benzonitrile, 1,2,4-trimethylbenzene, 1,2,3-trimethylbenzene, acetophenone, nonanal, and naphthalene from different suppliers Fisher Scientific (Pittsburgh, PA), Sigma-Aldrich (St. Louis, MO), TCI America (Tokyo, Japan), Acros (New Jersey, USA), and Aldrich (Milwaukee, WI), were used to prepare stock solutions. The purity of the compounds was equal or larger than 97.0 % except for the following compounds: 1,2,3-trimethylbenzene (90.0 %), benzonitrile and nonanal (95.0 %).

The stock solutions were prepared in-house to perform quantitative determinations. External calibration curves were performed to quantify the organic compounds. Calibration curves for GC-MS analysis were prepared by direct liquid injection with the aid of an autosampler, by direct spike in the CMV, and by headspace extraction with the CMV. The calibration curves with direct liquid injections were performed with a 1 μL volume of 1.0 ppm to 30 ppm mixture solutions. Calibration curves for CMV-GC-MS analysis were prepared by spiking 1 μL directly on the CMV device of the standard solutions prepared in the range of 5.0 ppm (ng μL^{-1}) to 30 ppm.

The headspace calibration curves were prepared by spiking 1 μL inside a quart can (~0.946 L) followed by extraction with the CMV device. The standard solutions for

headspace extraction were prepared in the range of 5.0 ppm (ng μL^{-1}) to 300 ppm, according to the expected amount for each compound in polluted ambient air. All the solutions were prepared in methanol as the solvent.

For headspace extraction analyses with the CMV, 1 μL of the standard solution was spiked inside a quart can and the instrument signal was quantified against a calibration curve created by spiking 1 μL of solution on the CMV devices. Therefore, the unit for amount detected on the spiked CMV is reported in ng, which was calculated by multiply the volume spiked (1 μL) times the concentration of standard solution analyzed (ng μL^{-1}). The reported amount (ng) for the headspace calibration curve depends on the calculated extracted amount.

2.4.3 Sample preparation

Minimum sample preparation was required to perform analysis by CMV-GC-MS. A 1 μL sample was spiked on a Kimwipe (Kimberly-Clark Global Sales LLC, Roswell, GA) inside a quart can. The headspace extraction of VOCs was performed with a CMV device attached to a tube connected to a portable air sampling pump (Escort Elf Pump, Ocala, FL) operated at a constant flow of 0.2 L/min. After headspace extraction for 10 min, the volatile components adsorbed to the CMV were analyzed by GC-MS with the aid of the thermal separation probe (TSP).

Previous to analysis, conditioning of the CMV was performed by placing the CMV in an oven at 250 °C for 30 min. Then the CMV was desorbed in the GC-MS to assure that the device was clean from VOCs.

2.4.4 Data reduction and analysis

Data reduction and statistical analyses were performed with Microsoft Excel 2010 (v 14.0.7153.5000, Microsoft Corp., Redmond, WA), and MSD ChemStation data analysis software (v E.02.01.1177 Agilent Technologies, Santa Clara, CA).

2.5 Results and discussion

2.5.1 Development and optimization of CMV-GC-MS for the analysis of VOCs

The headspace extraction technique was first optimized for analysis of VOCs using standard compounds. The parameters optimized were equilibrium time, and sampling flow rate. The equilibrium time is a measure of the optimal time required to allow partitioning between the sample phase (liquid or solid) and the headspace (gas). Experimentally, the equilibrium time was determined by extracting replicates of the same amount of compounds after different equilibrium times. The plot of equilibrium time and integrated area should show a plateau on the area after equilibrium is reached.

A 1 µL of a 10 ppm mixture solution (10 ng of each compound) was spiked on a Kimwipe that was placed inside a quart can. The quart can was sealed using a red rubber sleeved stopper and left to stand (or equilibrate) at room temperature (20.0-21.0˚C). The same procedure was followed in 3 replicates for 30 s, 1 min, 5 min, 10 min, and 30 min equilibrium time. In order to extract the same sampling volume (2 L) from the quart cans, the headspace extraction time was fixed to 2 min and the sampling flow rate at 1 L/min. Equilibrium was reached within 1 min for the target compounds.

Further optimization of the equilibrium time was performed at 15 s, 30 s, 45 s, 1 min, 2 min, and 5 min. Figure 4 shows an example of the results obtained in the

optimization. There is not a significant change in amount extracted at these equilibrium times as expected because these compounds are highly volatile. As shown in the figure, benzene was not detected because the concentration (10 ppm) used for this experiment is very close to the limit of detection for this compound. The first optimization results were kept, and future studies were performed for 1 min equilibrium time.

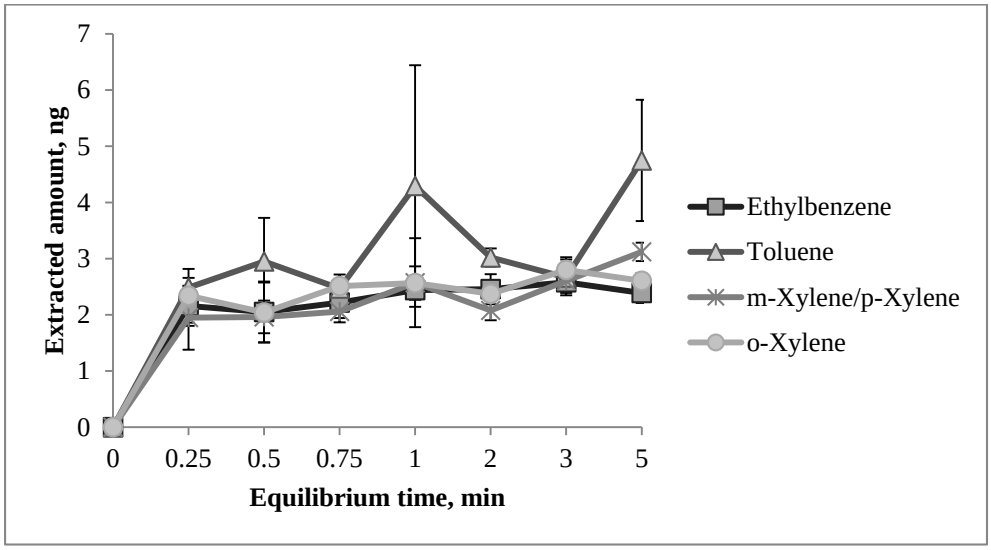

Figure 4. Equilibrium time determination of BTEX compounds at 1.0 L/min flow rate and 2 min extraction time

Following optimization of equilibrium time, the sampling flow rate was optimized as to reduce % breakthrough and extract the largest amount of analyte. Keeping sampling volume (2 L) constant, different flow rates were tested (0.2, 1, 2 L/min) at 10, 2, 1 min extraction time, respectively. Figure 5 shows an example of the BTEX compounds at different sampling flow rates. Error bars for all data points were graphed but because these are very small it is not possible to see in the figure. As shown in the figure, benzene

was not detected at 0.2 L/min at the concentration (10 ppm) used for this experiment and is very close to the limit of detection for the other sampling flow rate.

According to the results obtained in the sampling flow rate experiment for all the compounds 1 L/min and 2 L/min results were very similar. The extraction at 0.2 L/min resulted in higher integrated peak area for all the compounds.

A fast GC-MS method (14.5 min) was tested for the detection of 17 volatile organic compounds commonly found in the air of polluted environments. All compounds were detected with this method and were separated. A blank sample was analyzed between liquid injections to determine carry-over. None of the compounds were found to have carry-over using the selected GC-MS method, thus all the experiments were performed with the same method as described in section 2.4.1.1.

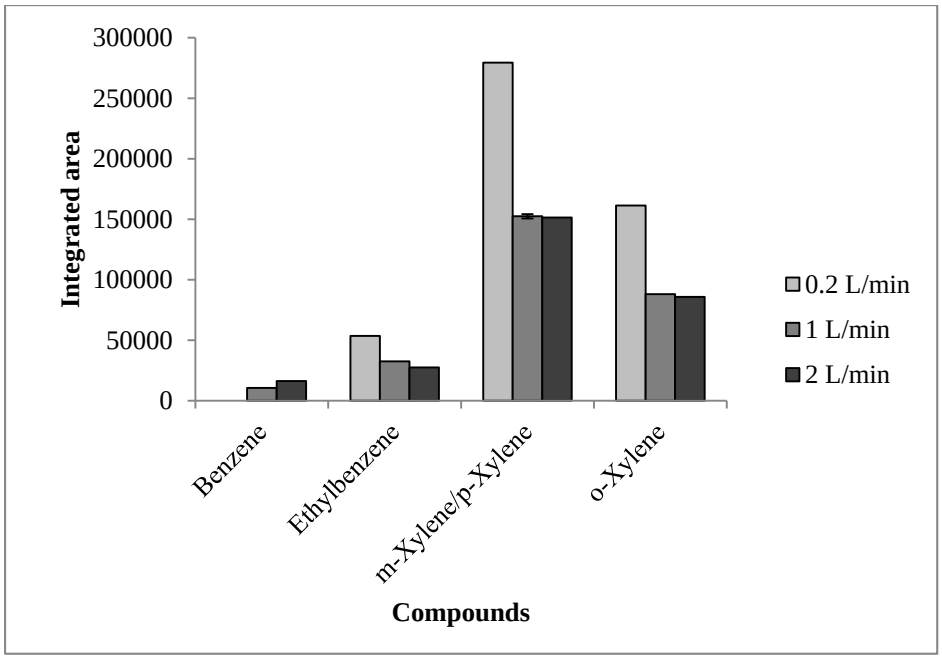

Figure 5. Sampling flow rate experiment showing BTEX compounds at a constant sampling volume (2 L) and different extraction times (10 min, 2 min, and 1 min)

2.5.1.1 Calibration strategies and the selection of the VOCs menu

To determine the validity of this method, compounds were selected with a wide range of boiling point temperatures (40.0°C-217.9°C) [Haynes 2015]. The list of compounds used for this study can be found in Table 2.

The retention time for each of the standard compounds was determined by performing automatic liquid injections with 20 ppm standard solutions for each individual compound. Only two compounds were observed to have the same retention time and mass spectra, m-xylene and p-xylene, thus joint identification and quantitation was performed for these compounds.

Calibration curves by liquid injection (autosampler) were generated with mixture solutions of the target compounds at different concentrations (1 ppm-25 ppm). Relatively good linearity of 0.865 or better was observed for all compounds. The lack of linearity for the calibration curves is thought to be a result of the expansion of the mixture solution in the injection port, which can result in sample loss.

Calibration curves by direct spike on the CMV were generated by spiking 1 µL of mixture solutions at different concentrations (1 ppm-25 ppm). The unit for amount detected is reported in ng, which was calculated by multiply the volume spiked (1 µL) times the concentration of standard solution analyzed (ng μL^{-1}). Good linearity of 0.969 or better was observed for all compounds.

Similarly, calibration curves by headspace extraction with the CMV were generated by spiking 1 µL of mixture solutions at different concentrations (1 ppm-300 ppm) in quart cans. An example of a headspace calibration curves for BTEX compounds is shown in Figure 6. Good linearity of 0.951 or better was observed for all compounds.

Table 2 shows the percent recovery for the extraction of compounds with CMV, which range from 4-23%, except for nonanal (0.3%).

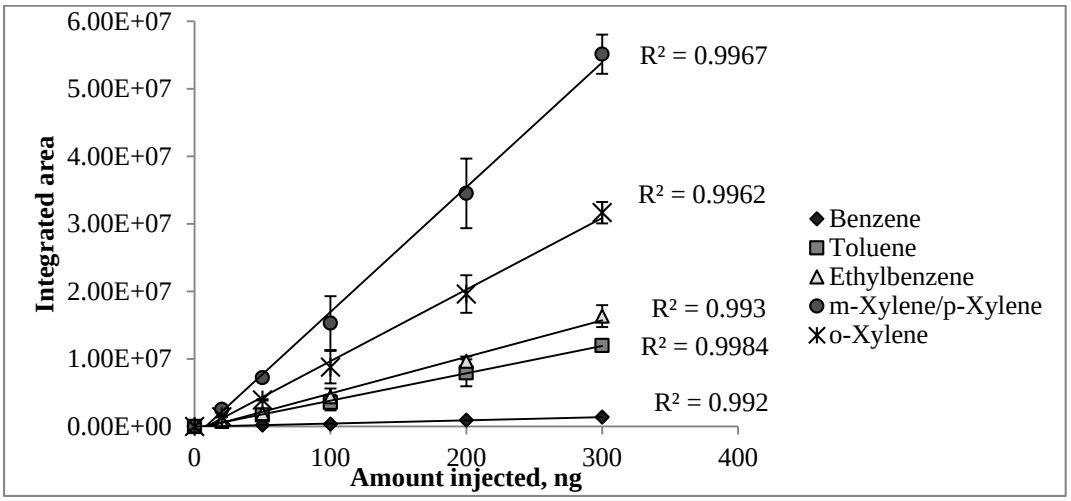

Figure 6. Calibration curves for BTEX compounds generated by headspace extraction at a sampling flow rate of 0.2 L/min at 10 min extraction time (2 L)

2.5.2 Figures of merit for CMV-GC-MS

The figures of merit for CMV extraction and analysis by GC-MS are summarized in Table 1. The compounds of interest show a linear response in the concentration range (5-300 ng) expected for VOCs in ambient air.

The method detection limits (MDL) for all compounds studied were determined for both the direct spike analysis on CMV and headspace extraction. Detection limits for each compound was determined by performing 10 replicates of blank samples and using Equation 1 (Section 2.1.2). It is worth mentioning that the reported MDL in Table 1 for headspace extraction calibration curves represent the minimal sample concentration that can be spiked on a can for the signal-to-noise ratio (SNR) of the response to be 3. The MDL calculated for headspace extraction with the method mentioned above resulted in

values in the range of 0.6-7.6 ng. Similarly, the calculated MDL for direct spike on the CMV yield values in the range of 0.2-2.9 ng. The MDL were confirmed by obtaining seven replicate measurements of a concentration close to the expected detection limit and multiplying it by 3.14, as specified in method TO-17 [EPA TO-17].

From all the compounds used in this study, calibration curves for methylene chloride, pyridine, and phenol were not created. These compounds were detected at a high spiked concentration (~500ppm), therefore the MDL and MQL were calculated using the method specified in the EPA method TO-17.

Table 1. Compounds list in order of elution time (t_R), quantifier and qualifier ions, and method limit of detection acceptability criteria for headspace extraction with CMV devices

			Qualifier ions		Direct spike on CMV			Headspace extraction with CMV			
Compounds	t_R min	Quantifier ion	Q1	Q2	MDL ng	MQL ng	%RSD	MDL ng	MQL ng	%RSD	%Recovery
Methylene chloride	2.61	84	49	86	4.1	14	25	10[b]	30[b]	23[c]	2.7[c]
Benzene	3.44	78	77	51	4.6	15	19	37	123	47	9
Pyridine	4.35	79	52	78	3.3	11	13	60[b]	187[b]	38[c]	10[c]
Toluene	4.54	91	92	65	2.9	10	23	17	55	33	16
Furfural	5.31	96	95	39	3.7	12	15	74	247	41	6
Ethylbenzene	5.67	91	106	77	2.0	6.6	11	33	110	28	23
m-Xylene/p-Xylene	5.77	91	106	77	3.0	10	15	23	75	26	16
o-Xylene	6.05	91	106	105	2.9	10	12	24	81	27	15
Benzaldehyde	6.87	105	106	77	2.5	8.4	12	52	173	17	15
Phenol	6.92	94	66	65	3.1	10	15	2.2[b]	6.1[b]	23[c]	0.6[c]
Benzonitrile	7.09	103	76	50	2.6	8.8	14	53	177	29	10
1,2,4-Trimethylbenzene	7.19	105	120	91	2.4	8.1	12	47	155	29	13
1,2,3-Trimethylbenzene	7.47	105	120	91	2.6	8.7	14	88	292	34	10
Acetophenone	7.88	105	77	120	2.8	9.3	11	83	276	29	4
Nonanal	8.15	57	67	81	5.6	19	9	95	315	29	0.3
Naphthalene	8.88	128	127	102	2.5	8.4	12	56	186	22	10

[a]Retention times (t_R), method detection limit (MDL) and method quantitation limit (MQL) for direct spike on the CMV and headspace extraction with the CMV, precision in the calibration curve at the middle concentration (15ng and 100ng), %recovery from headspace extraction at the middle concentration (100ng).
[b]MDL and MQL for this compounds were calculated as stated in the EPA method TO-17.
[c]Precision (%RSD) and %Recovery were calculated for these compounds at 500ppm.

2.5.3 Results for the performance criteria of CMV-GC-MS for detection of VOCs

The acceptability of the method to use CMV as a headspace extraction technique was established by following the criteria specified in the EPA TO-17. The criteria and respective equations were described above and include: method detection limit (MDL) of ≤ 0.5 ppbv (~5 ng), analytical precision of replicate measurements within 20%, precision for the distributed volume pair of 25% or less, and an audit accuracy of 30% or better for the expected concentration range 0.5-25 ppbv (5-300 ng). All criteria were followed to validate the method for headspace extraction with the CMV. Table 2 is a summary of the results obtained.

Table 2. Compounds list in order of elution time (t_R), quantifier and qualifier ions, and method acceptability criteria for headspace extraction with CMV devices

Compounds	t_R min	Quantifier ion	Qualifier ions Q1	Qualifier ions Q2	Breakthrough %	Analytical Precision %	Distributed volume pair %	Accy %
Methylene chloride	2.61	84	49	86				
Benzene	3.44	78	77	51	46	15		8.9
Pyridine	4.35	79	52	78				
Toluene	4.54	91	92	65	35	20	12	8.2
Furfural	5.31	96	95	39		4		9.2
Ethylbenzene	5.67	91	106	77	27	3	16	7.4
m-Xylene/p-Xylene	5.77	91	106	77	22	18	17	8.2
o-Xylene	6.05	91	106	105	21	14	11	8.3
Benzaldehyde	6.87	105	106	77	10	1	11	8.4
Phenol	6.92	94	66	65				
Benzonitrile	7.09	103	76	50	7	2	36	8.9
1,2,4-Trimethylbenzene	7.19	105	120	91	12	3	6	8.6
1,2,3-Trimethylbenzene	7.47	105	120	91	15	3	10	8.8
Acetophenone	7.88	105	77	120	28	42	34	9.5
Nonanal	8.15	57	67	81	19	60	1.3	9.9
Naphthalene	8.88	128	127	102	5	18	10	8.9

[a]Retention times (t_R), breakthrough at 0.2 L/min sampling rate and 30ng of standards (below detection limit for compounds that a value is not reported), analytical precision in percent, precision for the distributed volume pair, and audit accuracy at 200 ng of standards.

The breakthrough was higher than the specified in the EPA TO-17 method (5%) except for benzonitrile (7%) and naphthalene (5%). Nonetheless, the compounds were detected at the expected detection limits.

As previously mentioned, the MDL for headspace extraction, calculated using the blank-can samples fall within the expected concentration (~5 ng) for most of the compounds studied. The MDL can also be estimated from the results reported in Table 1 using the %Recovery (~10%) for the target compounds.

The analytical precision was calculated for most of the compounds and the results are reported in Table 2. At concentrations of 200 ppm, the precision was in the range of 1-20% except for acetophenone (42%) and nonanal (60%). Therefore, this method may be fit-for-purpose for most applications.

The distributed volume pair precision is calculated by performing several measurements at different volumes. To calculate the precision of distributed volume pair, two different sampling volumes (2L and 3 L) were evaluated. The distributed volume pair precision obtained ranged from 1.3-17% for all compounds except for acetophenone (34%) and benzonitrile (36%). Factors that can affect the precision are artifact formation, and breakthrough of the compounds. Any of these factors is likely to occur since the can blanks show the presence of artifacts, and breakthrough for these compounds is greater than 5%. Finally, the % audit accuracy was within the expected range (30%) for all the compounds.

The experimental results demonstrate that the following compounds met all the EPA method TO-17 criteria: Benzene, toluene, furfural, ethylbenzene, m-xylene, p-xylene, o-xylene, benzaldehyde, 1,2,4-trimethylbenzene, 1,2,3-trimethylbenzene, and naphthalene.

The EPA headspace method parameters were also tested for compounds that can be detected in the headspace of smokeless powders. Smokeless powders are present in the

propellant of ammunition and can provide additional information in the detection of gunshot residues (GSR). In Chapter 3, a description of gunshot residues and the significance of this forensic evidence will be introduced. Some of the compounds found in the headspace of smokeless powders are: Nitroglycerine (NG), 2,4-dinitrotoluene (2,4-DNT), and diphenylamine (DPA).

For the detection of volatiles from GSR using the CMV method was found to meet all the EPA performance criteria of 1) method detection limit of 0.5 ppbv or less, 2) analytical precision of 20%, 3) precision for distributed volume pair of 25% or less, and 4) an audit accuracy within 30% for NG. For DPA, only the % audit accuracy was not met at 40%, and for 2,4-DNT, the distributed volume pair percent (32%) and the % audit accuracy (40%) was not met. One of the reasons for not meeting the EPA criteria is the high breakthrough for the target compounds, which is in the range of 23-34%.

2.5.4 Results for headspace extraction of ambient air samples

Using the optimized sampling flow rate (0.2 L/min) and 10 min extraction time, indoor air from three different rooms was extracted in 3 replicates. Indoor air was extracted from a chemistry research laboratory (918 ft^2), a classroom in a building (1694 ft^2), and a hair and nail salon (1053 ft^2) [FIU, Modesto A. Maidique Campus, Building Plans]. The extraction in the research laboratory was performed in one half of the room. The portable pump and the CMV were placed on top of a table and the CMV was positioned in an upward direction for the 10 min extraction time. The air extraction in the classroom was performed in one of the corners of the room. The portable pump and the CMV were placed on top of a desk and the CMV was positioned in an upward direction

for the 10 min extraction time. There was negligible air turbulence in the extraction process for the laboratory and the classroom, because there was no movement of people in the area selected for extraction. The air extraction in the hair and nail salon was performed towards the middle of the room between the hair and nail sections. The portable pump and the CMV were placed on top of a table and the CMV was positioned in an upward direction for the 10 min extraction time. There were several people constantly walking near (within 3 feet) the collection area, therefore it is expected that some air turbulence occurred other than the air conditioning cold air flow. All replicates were performed in the same location for each room. The extraction was performed at room temperature, which was below 21.0°C for all rooms.

The CMV devices were previously conditioned in the laboratory for 30 min at 250°C and were tested with the GC-MS to compare the background signal with the room signal. The CMVs were each wrapped in aluminum foil to transport to the room location. After collection the CMV was wrapped again the aluminum foil for transportation back to the laboratory and perform the analysis by GC-MS.

An example of the chromatograms obtained from the three rooms compared to the blank CMVs is presented in Figure 7. Confirmation of the compounds detected was performed by comparison of the mass spectra with the NIST library and by injecting a 10 ppm standard solution to determine the retention time of each compound. The compounds that were compared to the NIST library only were: ethyl ester methacrylic acid and butyl ester acetic acid. The peaks shown in Figure 7 can be identified with the identification number used in Table 3.

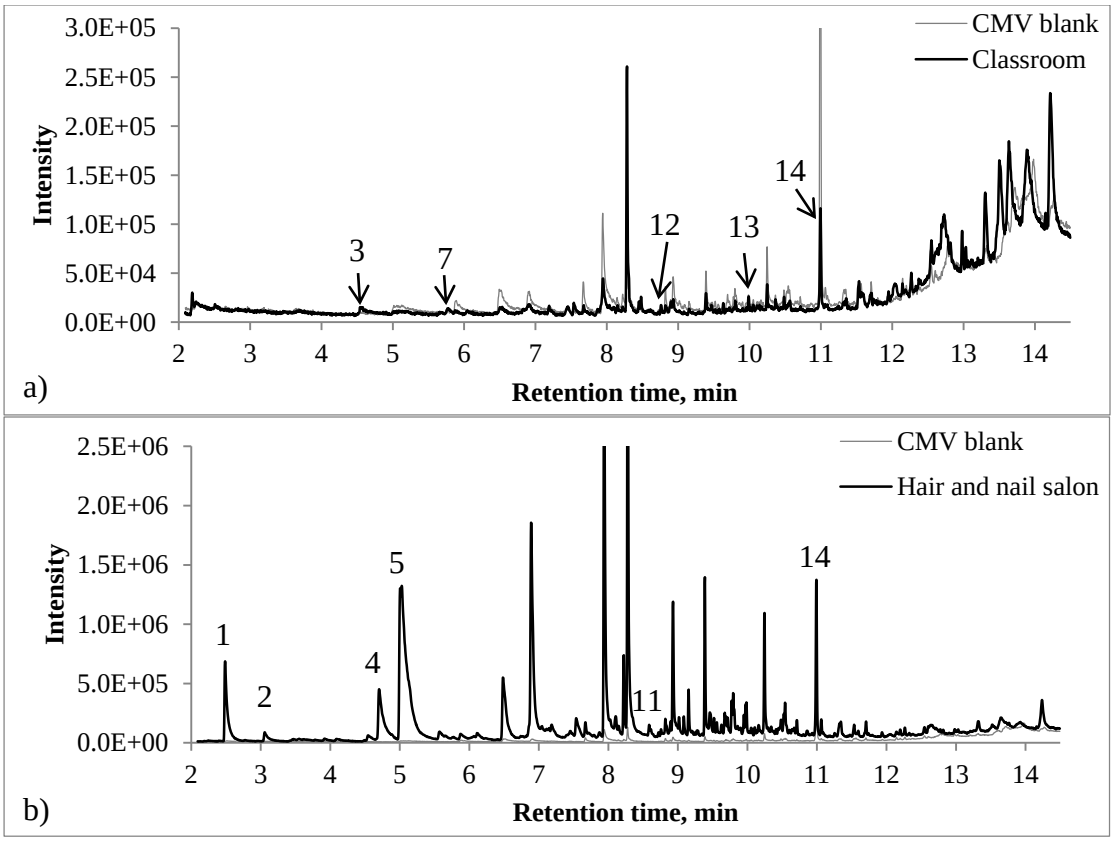

Figure 7. Example of the chromatogram obtained from the headspace extraction of indoor air from a) a classroom, and b) a hair and nail salon compared to the blank CMV

Table 3 is a summary of the compounds detected in the air for the three different rooms and the amount extracted for compounds for which a calibration curve was previously constructed. In the laboratory, benzaldehyde was present in one of the replicates. All the compounds detected were at or just above the method detection limit. In the classroom samples, toluene and m-,p-xylene were detected at similar concentrations compared to the lab samples. In the hair and nail salon, the signal intensities were higher than for the other rooms. Benzaldehyde was detected in one sample, and the concentration detected was significantly higher compared to the laboratory samples.

Table 3. Compounds detected in indoor air samples taken from a laboratory, a classroom and a hair salon using the optimized sampling flow rate (0.2 L/min)

No.	t_R min	Laboratory	Amount ng	Classroom	Amount ng	Hair and nail salon	Amount ng
1	2.48					Acetone	-
2	3.05					Ethyl acetate	-
3	4.55	Toluene	1.7	Toluene	1.6		
4	4.70					Ethyl ester methacrylic acid	-
5	5.00					Butyl ester acetic acid	-
6	5.67	Ethylbenzene	0.9				
7	5.77	m-,p-xylene	0.4	m-,p-xylene	0.4		
8	6.05	o-xylene	0.6				
9	6.84	Benzaldehyde	0.2			Benzaldehyde	19
10	7.20	1,2,4-trimethylbenzene	0.2				
11	8.58					Camphor	-
12	8.89			Decanal	-		
13	10.06			Dodecanal	-		
14	10.99			Diethyl phthalate	-	Diethyl phthalate	-

To corroborate the detection of some compounds in indoor air from the hair and nail salon, the material safety data sheet (MSDS) for cosmetic products used in salons was accessed. All the compounds detected in the hair and nail salon were found to be present in at least one of the commercial cosmetic products.

2.6 Conclusions for the analysis of VOCs in ambient air

Current methods for the analysis of VOCs in polluted air consist of lengthy sampling times (> 1 hr) following long GC methods. The proposed method provided fast sampling and detection of VOCs using a novel technique, CMV-GC-MS. The CMV extraction method has been shown to be a fast and sensitive technique for the headspace extraction of organic volatiles present in the air. In addition, headspace extraction calibration curves demonstrated the ability to perform quantitative results through this sampling method.

The applicability of CMV devices for the analysis of VOCs in ambient air was demonstrated by following the criteria of the EPA compendium method TO-17. The

criteria specified in the EPA method TO-17 consisted of: method detection limit (MDL) of ≤ 0.5 ppbv (~5 ng), analytical precision of replicate measurements within 20%, precision for the distributed volume pair of 25% or less, and an audit accuracy of 30% or better for the expected concentration range 0.5-25 ppbv (5-300 ng).

The experimental results were obtained by spiking 1µL sample volume in quart cans and performing headspace extraction with the CMV devices. It was demonstrated that the breakthrough could be potentially minimized by using a lower sampling flow rate. Nonetheless, the CMV provided enough sensitivity to comply with expected limits of detection (~5 ng). The majority of the compounds met the performance criteria specified in the EPA method TO-17.

In addition to the four criteria, the EPA method TO-17 also recommends a percent breakthrough of 5% or less. A percent breakthrough of 5% is considered acceptable for quantitative analysis. Nevertheless, the presented work was intended to demonstrate fit-of-purpose rather than performing quantitative analysis.

As proof of concept, headspace extraction with the CMV was performed in three different rooms. These samples were extracted directly from open indoor air without the use of cans. Some of the compounds evaluated in this study were found to be present at the limit of detection in the laboratory. In addition, benzaldehyde was present in significant amounts in the hair and nail salon. Other compounds detected in the hair and nail salon (i.e., acetone, ethyl acetate) were corroborated by accessing the MSDS of commercial cosmetic products. The suitability of the CMV for indoor air monitoring was demonstrated in this study.

The major advantages of CMV devices are the ability to use large sampling flow rates with extraction times of 2 min or less and cost efficiency, which make these devices disposable if multiple uses are not desired. Unlike expensive sorbent tubes, the CMV can be used multiple times without losing extraction capabilities and the absorbent material in CMV allows for the analysis of a wide range of compounds.

3 ANALYSIS OF ORGANIC AND INORGANIC COMPONENTS OF GSR

3.1 Utility of chemical analysis for GSR identification

The chemical analysis of materials is an important aspect of forensic examination because it can provide additional information and confirmation in the investigation of a criminal case. Gunshot residue evidence represents a challenging and complex matrix because it contains both inorganic and organic components.

Traditionally, GSR analysis consisted of identifying particles with a round morphology and performing elemental analysis on those particles. Wolten et al., were the first to establish an elemental profile for the round particles found in GSR samples. The elemental profile consisted of the presence of barium (Ba), lead (Pb), and antimony (Sb). In addition, chromophore tests were adopted to determine the presence of these elements in GSR, but color tests are presumptive and may produce false results because of interferences or erroneous color perception by the analyst. Presumptive tests are discussed in more detail in a later section (Section 3.3.2 and 3.3.3).

In most forensic cases involving firearms, the main question to answer is who fired the weapon. Analysis by SEM-EDS can provide this information with certain degree of confidence as long as Ba, Pb, and Sb are all present in the sample. Particles containing these elements are reported to be characteristic of GSR presence. Other classifications exist when particles contain only two or one of the target elements in combination with other elements, those particles are reported to be consistent with GSR presence [ASTM E1588-10]. Consequently, consistency does not provide strong evidence of GSR presence and additional evidence is needed.

Another reason not to rely on elemental analysis alone is the potential to find particles with similar composition, which can lead to false positive results [Martiny et al., 2008]. Several studies have indicated the presence of target elements in environmental particles, and trace amounts of these elements on the hands and clothing of individuals working in different professions, such as automobile mechanics, and workers exposed to pyrotechnics [Garofano et al., 1999; Brożek-Mucha et al., 2009; Dalby et al., 2010; Brożek-Mucha et al., 2015].

As a result, current analytical approaches seek to broaden the elemental profile of GSR as well as to characterize GSR by organic composition. The analysis of organic compounds in GSR has been previously suggested to serve as complementary information, which can provide improved identification and confirmation of the results [Benito et al., 2015]. The characterization of organic components in GSR is of particular importance in forensic examination because of current trends to minimize the use of toxic elements, such as Pb, in the manufacture of ammunitions.

In this project, the chemical composition of GSR will be evaluated to identify additional target components that will aid in the identification of residues on the hands of shooters. For this purpose, it is important to first determine possible sources and components in the ammunition and firearm that can contribute to the chemical composition of GSR.

3.2 Chemical components found in firearms and ammunition that may contribute to GSR composition

Firearms are generally classified as handguns, rifles, and shotguns. In general, firearm discharge consists of pulling the trigger which will make the firing pin strike the primer cap of the ammunition and ignite the primer mixture in the cartridge. The high temperature (1500-2000 °C) and pressure (104 kPa) produced by the chemical reaction in the cartridge melts the primer mixture, which is sensitive to impact or electric shock [Flynn et al., 1998]. In turn, the ignition of the primer causes the ignition of the smokeless powders in the cartridge. A second rapid increase in temperature and pressure in the cartridge produces an explosion that will propel the bullet forward through the barrel and out of the firearm muzzle [Dalby et al., 2010]. An opening on the barrel (ejection port) is used to remove the spent cartridge from the firearm.

A cloud of particles and combustion material produced by the discharge explosion are ejected through the openings in the firearm. The high temperatures reached during the discharge exceeds the temperature of vaporization for elements such as barium (1140 °C), lead (1620 °C), and antimony (1380 °C) [Flynn et al., 1998]. Therefore, once the inorganic particles are outside the firearm, rapid recombination and condensation occurs at the lower temperatures in the environment. Similarly, the inefficient combustion reaction during the discharge generates particles of unburnt and partially burnt smokeless powders, which are also ejected and deposited around the area of discharge [Flynn et al., 1998]. Differences in the assembly and openings in the firearms affect the distribution of the particles and combustion materials in the surroundings [Schwoeble 2000].

Gunshot residue (GSR) or firearm discharge residue (FDR), are the terms used to describe the particles ejected from the firearm after discharge. Gunshot residue is mainly composed of elemental components from the primer mixture as well as partially burnt and unburnt smokeless powder, which escape from the openings of a firearm after discharge. Typically, the GSR abbreviation has been used to describe particles with inorganic composition and FDR describes particles with organic composition. In this work, GSR is the term used to collectively describe the target particles with either inorganic or organic composition. When necessary the terms organic gunshot residue (OGSR) or inorganic gunshot residue (IGSR) will be utilized.

3.2.1 Chemical contribution from the ammunition

The discharge of a gun is triggered by the ignition of energetic material in the cartridge through a process called deflagration. Deflagration is when the explosion is caused by ignition of a cold material through heat transfer. The energetic material that is used in ammunition varies among weapon type as well as the mixture in primers, igniters, and propellants. For instance, small caliber ammunition (<40 mm) are discharged with the ignition of the propellant by the primer. The chemical composition of the propellant mixture is designed to achieve the desired projectile motion, and to aid the accurate transport of the projectile to the target over a specified distance. Similarly, the primer mixture should be sensitive enough to activate with the percussion force, propagate the ignition to the propellant, and perform these tasks in an efficient manner depending on the mechanism of the weapon [Kirchner et al., 1993].

3.2.1.1 Primer

The primer mixture is encapsulated in the primer cup, which is commonly plated with nickel to resist corrosion and to provide a harder surface that will be in contact with the groves of the barrel [Brożek-Mucha et. al., 2007].

The primer mixture consists of the initiating explosive, oxidizing agent, fuel, and sensitizer [Meng et. al., 2007]. The mixture components can vary by manufacture and ammunition type (i.e., pistol, rifle, or shotgun). The most commonly known primer is Sinoxd®, which contains lead styphnate, antimony sulfide, and barium nitrate. Other primer mixtures were later developed to address environmental and health hazards, these include: Sellier®, Bellot®, Prage®, and Sintox®, a primer tagged with specific elements to use in ammunition for police in European countries [SWGGSR guidelines].

Inorganic compounds that may be present in the primer mixture include: aluminum sulfide (Al_2S_3), antimony (Sb) compounds, barium (Ba) compounds, boron (B), calcium silicide ($CaSi_2$), copper thiocyanate (CuSCN), gold (Ag), ground glass, lead (Pb) compounds, magnesium (Mg), mercury (Hg) compounds, potassium (K) compounds, Prussian blue [$Fe_7(CN)_{18}$], silicon (Si), sodium (Na) compounds, strontium nitrate ($Sr(NO_3)_2$), sulphur (S), tin (Sn), titanium (Ti), zinc peroxide (ZnO_2), and zirconium (Zr) [Dalby et al., 2010 Review]. These inorganic compounds may contribute to the composition of GSR. However, the components traditionally targeted are barium (Ba), lead (Pb), and antimony (Sb). Barium is added to the primer mixture as barium nitrate ($Ba(NO_3)_2$), or barium peroxide (BaO_2), Pb as lead azide ($Pb(N_3)_2$), lead dioxide (PbO_2), lead nitrate ($Pb(NO_3)_2$), lead styphnate ($C_6HN_3O_8Pb$), or lead thiocyanate

($Pb(SCN)_2$), and Sb is added as antimony (V) sulfide (Sb_2S_5), antimony sulfite ($Sb_2(SO_3)_5$), or antimony trisulfide (Sb_2S_3) [Dalby et al., 2010].

After firearm discharge, recombination of elemental composition occurs by condensation and rapid cooling in ambient temperatures. The target elements (Ba, Pb, and Sb) can be found together in the form of spherical particles that are a few microns in diameter (0.1-100 μm) [Flynn et al., 1998; López-López et al., 2012].

3.2.1.2 Propellant

The propellant is mainly composed of solid smokeless powders, which are also known as grains. Partially burnt and unburnt smokeless powders originate from the ammunition through an inefficient combustion process during firearm discharge. Therefore, the presence of characteristic organic compounds from smokeless powders can provide vital information in the detection of GSR.

Smokeless powders are characterized depending on its energetic material preparation: single-based prepared by dissolving nitrocellulose (NC) in ether and methanol, double-based prepared by dissolving nitrocellulose in nitroglycerine (NG), and triple-based prepared by dissolving nitrocellulose in nitroglycerine with nitroguanidine [Kirchner et al., 1993]. Triple based smokeless powders are rarely used in small caliber ammunition (i.e., handguns, rifles, and shotguns), thus discussion will be limited to propellants made of single and double based smokeless powders.

The propellant also contains other additives to enhance the efficiency of the energetic material to burn the smokeless powder and create an explosion as well as to increase the shelve-life of the ammunition. For instance, the main organic compounds

detected in smokeless powders are diphenylamine (DPA), a stabilizer to prevent accumulation of the decomposing materials, and ethyl centralite (EC), a deterrent to slow the burning rate of the smokeless powder. Other additives include dinitrotoluene (DNT) isomers (i.e., 2,3-DNT, 2,4-DNT, and 2,6-DNT), methyl centralite (MC), and dialkyl phthalates [Andrasko et al., 1998; Reardon et al., 2000; Weyermann et al., 2009].

Inorganic materials can also be added to neutralize decomposition products in the propellant, such as calcium carbonate ($CaCO_3$). Decoppering additives to prevent the buildup of copper in the barrel include: tin dioxide (SnO_2), bismuth (Bi) compounds, and lead compounds. Flash reducers that use potassium (K), such as potassium chloride (KCl), reduce the brightness of the muzzle flash during discharge. In addition, wear reduction additives prevent the erosion of barrel liners. These additives could include: wax, talc [$Mg_3SiO_4O_{10}(OH)_2$], and titanium oxide (TiO_2) [Kirchner et al., 1993].

Following the discharge, combustion products from the propellant are created such as carbon dioxide, water, carbon monoxide, hydrogen, nitrogen, and nitric oxide. If found at high concentrations (>50 ppm), nitric oxide is converted to nitrogen dioxide in the presence of oxygen in ambient air from the high temperature combustion of the propellant. On the other hand, ammonia can be formed from the combination of nitrogen from compounds containing nitrogen groups and hydrogen by the following reaction:

$$N_2 + 3H_2 \rightarrow 2NH_3 + 22.0\ kCal$$

Similarly, sulfur dioxide may form when antimony sulfide is used in the primer mixture, and potassium sulfate, used as a flame retardant in propellants, are oxidized [Kirchner et al., 1993].

3.2.1.3 Casing and bullet

The general components of ammunitions are shown in Figure 8. The primer and propellant are encapsulated in the casing, which is a metal cylinder. A smear of this metal casing may be removed at high temperatures during the firearm discharge [Dalby et al., 2010]. A projectile or bullet made of lead is placed in front of the casing. The bullet core is often made of lead and antimony alloy because the high melting point of antimony allows the bullet to be fired at a faster rate. Antimony trioxide is a combustion byproduct of the antimony in the bullet and primer mixture (antimony sulfide) [Kirchner et al., 1993]. If the bullet is jacketed or covered with a different element, that element may also be detected in GSR. The bullet cases are often made of brass, which contain elements such as zinc and copper to improve performance [Kirchner et al., 1993].

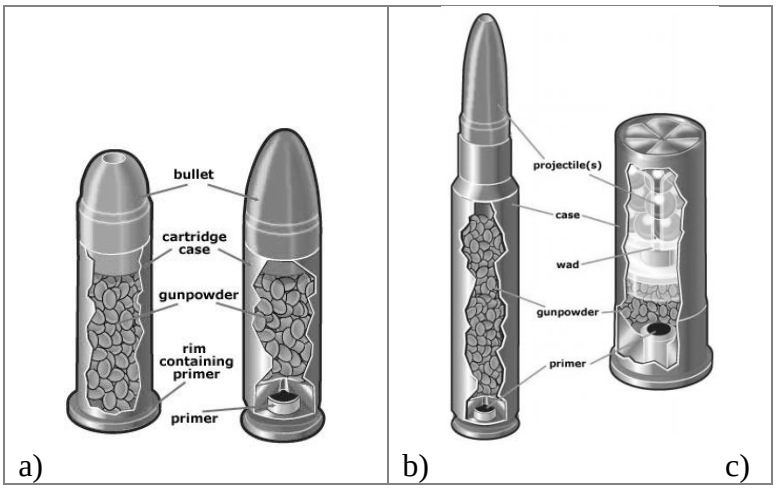

Figure 8. General components of a) pistol, b) rifle, and c) shotgun ammunition <https://www.handgunsafetycourse.com/images/drawings/ammo-handgun.jpg>

3.2.1.4 Non-toxic ammunition

The establishment of Ba, Pb, and Sb as the target elements for the presence of GSR was first introduced by Wolten. However, this elemental profile is applicable when analyzing primer mixtures such as SINOXID primers, which contain Ba, Pb, and Sb in its composition. Other primer mixtures can be lead-free or antimony-free, which may challenge the discrimination of GSR particles by the ASTM method [SWGGSR guidelines]. An example of environmental friendly ammunition manufacturers is the Brazilian Cartridge Company (CBC). The ammunition produced contains a primer mixture with metals such as, Ti, Cu, and Zn. The bullets for these ammunitions are jacketed to prevent contamination of Pb [Vanini et al., 2014].

According to the ASTM E1588-10, lead-free or non-toxic ammunition contains primers that generate particles characteristic of GSR presence with the following elemental composition: 1) Gadolinium (Gd), titanium (Ti), zinc (Zn); and 2) Gallium (Ga), copper (Cu), tin (Sn). Particles that are consistent with GSR are composed of Ti, Zn and other elements such as, Al, Si, Ca, Cu, or Sn. Another element that has also been reportedly found in GSR is Sr [ASTM E1588-10].

3.2.1.5 Environmental particles

Debates around the choice of the elemental profile for GSR continue to exist as more studies are demonstrating the possibility of finding one or more of the target elements in other matrixes. For instance, trace amounts of one or all of these elements can be detected on the hands and clothing of individuals involved in certain professions such as automobile mechanics, and people working with fireworks [Garofano et al.,

1999; Brożek-Mucha et al., 2009]. Also, particles found in the environment have similar elemental compositions to GSR [Dalby et al., 2010]. Moreover, ammunition similar to the ones manufactured at CBC does not produce spherical particles after discharge like the ones expected to be found in GSR [Vanini et al., 2015]. Therefore, just relying on the morphology of the particles and the presence of Ba, Pb, and Sb can generate false positive or false negative results [Martiny et al., 2008].

3.2.2 Chemical contribution from the firearm

Other compounds and elemental particles come from different parts of the firearm. For instance, the iron composition in GSR mainly comes from the firearm barrel and its effect of wear from heat induced erosion [Kirchner et al., 1993]. Similarly, the lubricants used to clean the weapon can be detected in the particles collected. A comprehensive list of elements and organic compounds found in GSR has been previously reported [Dalby et al., 2010].

3.3 Forensic examination of GSR

The forensic examination of GSR can present several challenges to the analyst due to the low availability of characteristic particles, and the loss of evidence through secondary transfer. In general, gunshot residue can be found on any surface in the area of the firearm discharge as well as on the person who fired the weapon, and any person in the vicinity. The distribution of the particles depends on the type of weapon and the ammunition used [Schwoeble 2000].

Forensic analysts have been able to collect GSR particles from different locations on a person such as clothing, hair, and hands [Weber et al., 2014]. In addition, several studies have confirmed the presence of GSR particles on a person close to the discharge, who is not the suspect. Also, secondary transfer of GSR particles is possible through a hand shake or by handling of the weapon [Brożek-Mucha et al., 2014; French et al., 2015]. Therefore in a criminal case, the presence of GSR on a person does not necessarily indicate the culpability of the person, but is definitive evidence that the person was present in the area of the discharge, or had direct contact with the shooter or the weapon.

In the following subsections, an overview of the different analytical techniques for the analysis of inorganic and organic components in GSR will be described. A brief discussion is presented on the advantages and disadvantages of current techniques and the importance of developing new strategies for the analysis of GSR.

3.3.1 Sample collection from the hands of a person

Sample collection from the hands of shooters has been widely investigated to determine the best location for sampling. The most common areas for sample collection on the hands include: palm, back, thumb, and the area of the hands that is in close proximity to the weapon as shown in Figure 9 [Vanini et al., 2014]. However, the collection of GSR is challenging because of the loss of particles through time. There are several factors that may contribute to particle loss such as washing hands, rubbing hands against other surfaces, putting hands inside pockets, or handcuffing hands behind back during arrest [Jalanti et al., 1999]. The times reported for GSR persistence on the hands of shooters range from 1-48 hours and depend greatly on experimental design [Jalanti et

al., 1999]. Likewise, casework studies suggest that it is possible to find GSR on the hands of shooters for longer period of times than those reported in laboratory experiments [Jalanti et al., 1999].

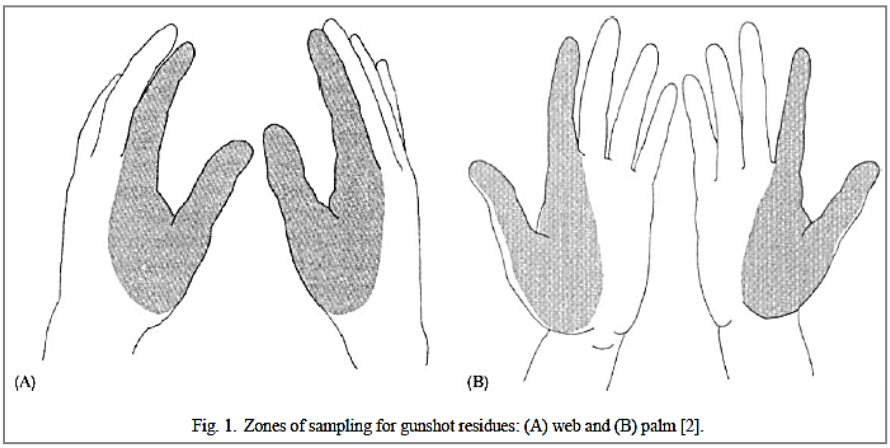

Fig. 1. Zones of sampling for gunshot residues: (A) web and (B) palm [2].

Figure 9. Common areas for sample collection of GSR from the hands of a person [Morales et al., 2004]

Sample collection efficiency to obtain the greatest number of particles has been investigated by several methods. The collection method may vary depending on the desired analytical technique. The most commonly used methods for the collection of GSR on the hands of shooters include: swabbing, dabbing with carbon adhesive mounted in an aluminum stub, and tape lifting [Goode et al., 2002; Dockery et al., 2003; Dalby et al., 2010; Brożek-Mucha et al., 2014; Benito et al., 2015].

Swabbing consists of rubbing the skin of the hands of a person using a commercially available applicator (or stick) with scrubbing material, such as cotton, at the tip. The swabbing can be performed dry or by previously moistening the swab material with a solvent. Studies have been performed to determine collection efficiency

using dry swabs versus moistened swabs. The results indicate that a greater number of particles can be collected using moistened swabs [Dalby et al., 2010]. In addition, Reid et al., suggest the use of swabbing if analysis of propellant is needed [Reid et al., 2010].

Several solvents have been utilized to collect GSR from the hands of shooters. The choice of solvent depends on the target analyte, organic or inorganic components in GSR, and on the analytical technique to be used. There are organic and inorganic solvents, usually organic solvents are used to collect the greatest amount of GSR with organic composition and inorganic solvents are used to collect the greatest amount of GSR with inorganic composition. The organic solvents that have been studied for GSR collection include: methanol, EDTA solution, and acetone [Vanini et al., 2014; Benito et al., 2015]. The inorganic solvents that have been studied for GSR collection include: water and nitric acid [Dalby et al., 2010].

Several studies reported the use of tape for collection of GSR on the hands of shooters [Dalby et al., 2010; Vanini et al., 2014]. A study was conducted in which 4 different tapes were tested for efficiency in collecting GSR particles [Vanini et al., 2014]. A previous study looked at eight different types of tape material [Dalby et al., 2010].

The most widely used method for sampling GSR is dabbing with carbon adhesive mounted on an aluminum stub because it is the method used for analysis by SEM-EDS. Dabbing with carbon adhesive consists of continuously pressing the adhesive onto the hands of the subject [Goode et al., 2002; Dockery et al., 2003; Brożek-Mucha et al., 2014; Benito et al., 2015; French et al., 2015]. The number of times that the carbon adhesive is pressed against the skin varies among studies, ranging from 20-100 times [Brożek-Mucha et al., 2014; Benito et al., 2015; French et al., 2015]. One of the

disadvantages of this technique is that skin debris or fibers can also get stuck on the adhesive and may mask the presence of GSR particles [Flynn et al., 1998].

3.3.2 Analysis of inorganic components in GSR from the hands of shooters

Gunshot residue particles containing inorganic components are generated after the firearm discharge by a process known as condensation. During discharge, the temperature in the barrel of the firearm exceeds the vaporization temperature of elements present in the ammunition such as Ba (1140°C), Pb (1620°C), and Sb (1380°C). When these particles are ejected, rapid cooling occurs due to the lower temperatures in ambient air. Thus, elements recombine during this process forming spherical particles with a unique elemental composition (Ba, Pb, and Sb). Recombination of more than one of these spherical shaped particles can occur, and a more irregular particle shape is observed. For this reason, a spherical morphology alone is not an indication of GSR particles [Grima et al., 2012]. Particle size can range between 0.1-100 μm, but most are found to be less than 10 μm [Flynn et al., 1998; López-López et al., 2012; Moran et al., 2013].

A technique commonly used for rapid screening of elements characteristic of GSR presence is the use of chromophoric tests such as the sodium rhodizonate test and the Harrison and Gilroy test [Dalby et al., 2010; Vanini et al., 2014]. These tests are usually performed in targets to determine shooting distance, but can also be applied to swab samples as a screening method [Berendes et al., 2006; Martiny et al., 2008]. The development of the sodium rhodizonate test is attributed to Feigl and Suter but the use of this reagent was first published by Feigl in 1924 [Feigl et al., 1942]. The sodium rhodizonate test can detect the presence of Pb by forming a bright pink color [Feigl et al.,

1942; Vanini et al., 2014]. The Harrison and Gilroy test consists of first swabbing the hands of a suspect with a cloth moistened with hydrochloric acid (HCl), and then triphenylmethylarsonium iodide was added to allow visualization of Sb followed with the addition of sodium rhodizonate for the detection of Ba and Pb. However, this test proved to have very low sensitivity and is not widely used [Di Maio 1999; Dalby et al., 2010].

Nonetheless, color tests are presumptive and lack reliability. Disadvantages of color tests include high rate of false positive results as a result of positive reactions with other substances, and are dependent on each individual visual color perception [Silva et al., 2009; Vanini et al., 2014]. In addition, color tests react to the presence of target elements but cannot be used as a confirmation of GSR presence [Dalby et al., 2010; López-López et al., 2012]. Therefore, elemental analysis is more reliable and is necessary to confirm the presence of target elements in GSR samples.

The method of choice for elemental analysis of GSR is Scanning Electron Microscopy-Energy Dispersion X-ray Spectroscopy (SEM-EDS). This method provides good selectivity and relatively good sensitivity for this application as well as imaging capabilities for morphology studies. The analysis of GSR through this method consists of collecting the samples with carbon adhesive mounted on aluminum stubs. The aluminum stubs are then placed inside the chamber of the SEM-EDS and an automated program designed for GSR analysis is used to find particles characteristic of GSR presence. The automated program will mainly detect light round particles, indicative of heavy elements presence, and a specified size, usually equal to or larger than 0.5 μm. The automated program should be able to provide particle coordinates on the aluminum stub and a spectrum of electron energy vs. number of counts. Once the particle scanning process is

completed the analyst has to manually confirm that these particles are in fact from GSR material by going to the location of the particles and re-acquiring a spectrum if necessary. The analysis of GSR through this technique is mainly qualitative reporting the absence or presence of particles with the desired elemental composition and the number of particles detected in the sample.

To standardize the analysis method of inorganic particles in GSR by SEM-EDS, an ASTM method (E1588-10) was developed. The ASTM E1588-10 provides guidelines for sample preparation, area of sample to be analyzed, instrument parameters and operation, and data analysis [ASTM E1588-10]. The Scientific Working Group for Gunshot Residue Analysis (SWGGSR) also established guidelines similar to those found in the ASTM method (E1588-10). For the data analysis section, GSR particles are classified as: characteristic, consistent, or commonly associated with GSR. Particles that are characteristic of GSR presence contain a combination of Ba, Pb, and Sb. The combination of two of these elements is considered to be consistent with GSR presence. Finally, particles associated with GSR will contain one of the target elements (Ba, Pb, or Sb) together with other elements. Particles with that composition can be readily found in particles from other matrixes [ASTM E1588-10; SWGGSR].

The downside of GSR analysis by SEM-EDS is that although finding GSR particles in the sample can be automated, it can take several hours (6-8 hours) for a single sample to be analyzed [Grima et al., 2012]. Also, manual examination of particles requires a trained analyst and can also be time consuming [Vanini et al., 2015]. Another disadvantage of the use of SEM-EDS is the low resolution capability, which can result in the overlap of peaks in the spectrum for target elements. For instance, Pb peaks overlap

with sulfur (S) peaks, which can lead to the erroneous classification of a particle [SWGGSR].

In an effort to improve analysis of GSR from lead and lead-free ammunition types, other techniques have been employed for detection and characterization. These techniques include: Neutron Activation Analysis (NAA), Atomic Absorption Spectrometry (AAS), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), Raman Spectroscopy, Scanning Electron Microscopy-Energy Dispersion X-ray (SEM-EDS), Laser Induced Breakdown Spectroscopy (LIBS), and Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) or Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) [Brożec-Mucha et al., 2009; Michel et al., 2010; Kumar et al., 2011; Charles et al., 2011; Brożec-Mucha et al., 2011].

A fast, sensitive and commercially available technique for elemental analysis is Laser Induced Breakdown Spectroscopy, LIBS. To the best of our knowledge, few laser ablation studies have been performed for the detection of elements in GSR. Laser ablation techniques can contribute to the characterization of GSR components and provide accurate quantitative analysis. Studies using LIBS have been conducted on GSR samples collected from individuals that have fired a gun. In research publications, sample collection from hands is performed with a double tape on a stub used for SEM analysis and with tape lifting (3M 5490 PTFE) [Goode et al., 2002; Dockery et al., 2003]. Although only qualitative analysis was performed, the authors concluded that LIBS could be a potential technique for GSR discrimination [Goode et al., 2002]. Dockery et al., detected the presence of GSR from the hands of shooters by LIBS with Ba emission lines: 455.403 nm, 493.409 nm, 553.548 nm, 614.172 nm, 649.690, 649.876 nm, and

705.994 nm. A blank sample before shooting was also collected from the hands of the shooter and analyzed. The elements reported to be present were: Ca (422.6728 nm), Na (588.9950 nm and 589.5924 nm), and K (766.4911 nm and 769.8974 nm) [Dockery et al., 2003]. The principles of LIBS are described in Section 3.5.1 of this chapter.

Among the solution analysis techniques mentioned earlier, ICP-OES was reported to be sensitive enough for the detection of barium with limits of detection of 0.0008 μg/mL compared to 0.002 μg/mL by AAS, and also has a broader linear dynamic range [Koons et al., 1988]. For the purpose of this project, ICP-OES will be used as a confirmatory technique for the detection of inorganic components in GSR from swab samples. This technique was chosen because it is sensitive and produces a similar spectrum output as LIBS, thus allowing direct comparison of the results. The principles of ICP-OES are described in Section 3.5.2 of this chapter.

3.3.3 Analysis of organic components in GSR from the hands of shooters

Gunshot residue particles containing organic components are generated after the firearm discharge burning of the smokeless powders. During discharge, the temperature in the barrel of the firearm increases rapidly and burns the smokeless powder. Since this process is not fully completed a combination of unburnt and partially burnt smokeless powders are ejected from openings in the firearm. Additionally, other combustion products are created during the process. A comprehensive list of compounds detected in GSR was previously reported [Dalby et al., 2010]. Chemical characterization of smokeless powders using different techniques has been widely reported in the literature. The main volatile organic components detected in smokeless powders are nitroglycerine

(NG), diphenylamine (DPA), 2,4-dinitrotoluene (2,4-DNT), ethyl centralite (EC) [Joshi et al., 2011]. It has been demonstrated that the chemical composition of unburnt and partially burnt smokeless powders in GSR contain a similar composition to that of the bulk smokeless powder before the discharge [Burleson et al., 2009].

A technique commonly used for rapid screening of organic components in GSR is the use of chromophoric tests such as, paraffin test (dermal nitrate test), and the modified Griess test [Dalby et al., 2010; Vanini et al., 2014; Vanini et al., 2015]. Most of these tests are usually performed in targets to determine shooting distance, but can also be applied to swab samples as a screening method [Berendes et al., 2006; Martiny et al., 2008]. One of the first tests designed as an attempt to detect compounds indicative of GSR presence on the hands of shooters was the paraffin test. The paraffin test consists of coating the hand of a suspect in warm wax to create a cast. Once the cast cooled, it is removed from the suspect's hands and an acidic solution of diphenylamine is sprayed in the cast. A blue color in the cast is a positive test for the presence of nitrites (NO_2^-) and nitrates (ONO_2) [Vanini et al., 2015]. The modified Griess test should be performed before any other color test to avoid chemical interferences. A positive reaction results in a pink-violet azo dye color [Dalby et al., 2010].

Nonetheless, color tests are presumptive and lack reliability. Disadvantages of color tests include high rate of false positive results due to positive reactions with other substances, and are dependent on each individual visual color perception [Silva et al., 2009; Vanini et al., 2014]. Chromophoric tests that detect the presence of nitrites and nitrates are unreliable because there are multiple materials found to contain these functional groups, such as fertilizers [Hilton et al., 2010]. These color tests react to the

presence of functional groups but cannot be used as a confirmation of GSR presence [Dalby et al., 2010; López-López et al., 2012]. Therefore, organic analysis is more reliable and is necessary to confirm the presence of target organic compounds in GSR samples.

Currently there is not an established technique for the analysis and detection of organic compounds in GSR samples from case studies. In an attempt to develop a method for organic components analysis, several techniques have been applied for the detection of GSR on the hands of shooters including: headspace extraction of volatile compounds using Solid Phase Microextraction (SPME) and analyzed by Gas Chromatography (GC) coupled to different detectors such as Flame Ionization (FID), Thermal Energy Analyzer (TEA), Electron Capture (ECD), and Mass Spectrometry (MS). In addition, solvent extraction followed by High Pressure Liquid Chromatograghy-Mass Spectrometry (HPLC-MS) has been applied, as well as Capillary Electrophoresis (EC), Ion Mobility Spectrometry (IMS), and Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) [Burleson et al., 2009; Silva et al., 2009; Weyermann et al., 2009; Zeichner et al., 2009; Dalby et al., 2010; Brożec-Mucha et al., 2011; Arndt et al. 2012]. However, most of these techniques require time consuming sample preparation and are destructive; therefore no further analysis can be performed.

The aim of this work is to provide law enforcement with a fast detection method for GSR on the hands of suspects that could be potentially applied on the field with commercially available portable systems. Some of the techniques currently used in research for GSR analysis are already available in portable systems such as IMS and GC-MS.

3.3.4 Analysis of organic components in GSR from spent cartridges

The collection of GSR in spent cartridges is similar to that described for particles collected from the hands of shooters. Analysis of spent cartridges is performed for several purposes including: determination of time since discharge, characterization of residues, and to evaluate whether GSR on the hands of suspects can be traced back to the ammunition used.

To determine time since discharge and characterize the residues in spent cartridges, several techniques have been used such as SPME to GC-MS and IMS [Andrasko et al., 1999; Weyermann et al., 2009]. The SPME method consists of extracting the headspace of the spent cartridge to detect volatile organic compounds [Andrasko et al., 1998; Wilson et al., 2003].

3.4 Fundamentals and principles of GC-μECD

An Electron Capture Detector (ECD) is the detector of choice for the analysis of explosive compounds. The advantages of using this detector is the high sensitivity and selectivity for compounds containing the NO_2 functional group, its low maintenance requirements, and simplicity of operation since it does not require expensive vacuum pumps to operate. The disadvantage of the ECD is that it is not an identification technique because it does not provide any information about the molecule. The signal obtained with this detector can be compared to that of a standard compound in terms of retention time. Figure 10 shows the schematic of an ECD detector.

The principles of ECD are described in terms of the electronegativity of the compounds eluting from the analytical column. The signal response depends on the

ability of the compounds to form negative ions by capturing an electron [Sevcik 1975]. Thus, the more electronegative a compound is the greater the response for the ECD.

The typical ionization source in ECD is the beta emitter ^{63}Ni. The ionization process with ^{63}Ni consists on the continuous formation of electrons by the radioactive source. The electrons are then captured by the electronegative molecules and produce the formation of negative molecular ions [Sevcik 1975].

For the purpose of this work, ECD is particularly sensitive for the detection of explosive compounds containing NO_2 functional groups (e.g., nitroglycerine and 2,4-dinitrotoluene), which has an electron affinity of 3.9 eV [Sevcik 1975].

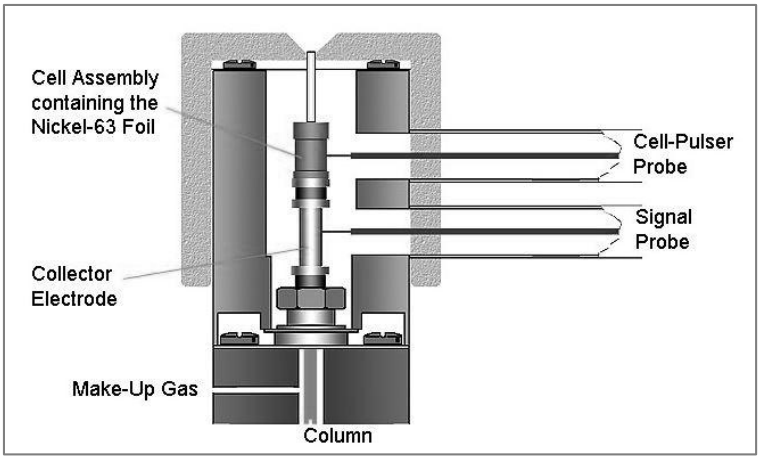

Figure 10. Schematic of an ECD detector with ^{63}Ni source
< http://www.queensu.ca/asu/instrumentation/gcmsfidecdnpd/ECDSch.JPG >

3.4.1 Coupling GC to multiple detectors through a Pneumatics Control Module

The coupling of GC to multiple detectors is possible today by the installation of a Pneumatics Control Module (PCM). There are many other advantages of having a PCM

including: changing analytical columns and performing maintenance on the GC unit without having to vent the MS unit.

The PCM consists of a board installed inside the GC oven, where the analytical column can be installed. Two or more other inlets are present to connect an uncoated glass capillary column to each of the detectors. Therefore, the flow coming from the analytical column can be distributed in any ratio to the detectors. In this way, not only are the substances separated by chromatographic methods but unequivocal confirmation can be obtained with two detectors. In the case of analysis of trace amount of explosives, the coupling of a MS and μECD can provide additional information by detecting the ions of interest and also obtaining a highly sensitive signal for the target compounds.

3.5 Fundamentals of LIBS and ICP-OES

Laser Induced Breakdown Spectroscopy (LIBS) and Inductively Coupled Plasma Optical Emision Spectroscopy (ICP-OES) are atomic emission spectroscopic techniques. Both techniques use a plasma as the light source and a spectrometer to sense the optical emission from the plasma, which creates an output or spectrum of intensity versus wavelength. Therefore, the information obtained with these techniques is very similar and can be used complementarily.

The main analytical difference between LIBS and ICP-OES is sample type. Analysis conducted by LIBS requires little to no sample preparation. Commonly, LIBS is used in the analysis of solid samples. On the other hand, typical analysis with ICP-OES requires the sample to be in liquid form. Nevertheless, laser ablation systems can be coupled to the ICP-OES for the analysis of solid materials.

Therefore, parameters optimization differs for LIBS and ICP-OES. In LIBS optimization is performed to obtain the formation of an efficient temporal plasma while in ICP-OES parameters are optimized for efficient transfer of an even micro-droplets of solution into the ICP-OES.

There are several advantages for the analysis of samples by LIBS, these include: direct analysis of the sample with minimal or no sample preparation, negligible sample consumption, and good sensitivity and selectivity.

3.5.1 Principles and capabilities of LIBS

A generic LIBS system contains the following components: the short pulsed laser, the focusing mirrors and lenses, the sample stage or cell, the collection system (lens, mirror, or fiber optic), the detection system, which includes the spectrometer to filter or disperse the light and the detector, and the computer to translate and process the data. Figure 11 shows a typical LIBS system with general components [Miziolek 2006].

The LIBS analysis process consists of detecting wavelengths originating from a micro-plasma created when a short pulse laser ablates the surface of the sample. In a LIBS system the most commonly used laser for forensic applications is the solid state nanosecond lasers, such as Nd:YAG lasers. These lasers can emit light at various wavelengths (1064, 532, 355, 266, or 213 nm) using a harmonic generator that changes the frequency of the laser [Miziolek 2006]. Other lasers such as the femtosecond lasers has many advantages including reduced fractionation, improved precision, and improved measurement accuracy.

In LIBS, the laser energy usually ranges from 10-100 mJ [Miziolek 2006]. The laser is first focused by going through different mirrors and lenses. A computer is usually used to adjust the focus of the camera that is providing the image of the sample. In turn, this process focusses the laser in or on the surface of the sample. It has been previously reported that focusing the laser a few millimeters into the sample produces better results [Fortes et al., 2010]. Once the laser reaches the sample, a small area is ablated and a micro-plasma is created. The micro-plasma produced contains a combination of electrons, excited atoms, and ions. When the excited electrons relax to the ground state, characteristic wavelengths are emitted. The first few seconds of emitted wavelength is known as a continuum, and no information can be obtained. As time passes, more of the atomic emission lines are observed and lastly the ionic emission lines can be perceived. In order to control the time at which information is collected the spectrometer is gated [Miziolek 2006]. The gate delay time can be optimized to achieve the best collection for either of the emission lines, or for a combination of the emission lines. The optimized gate delay will depend on the target analyte.

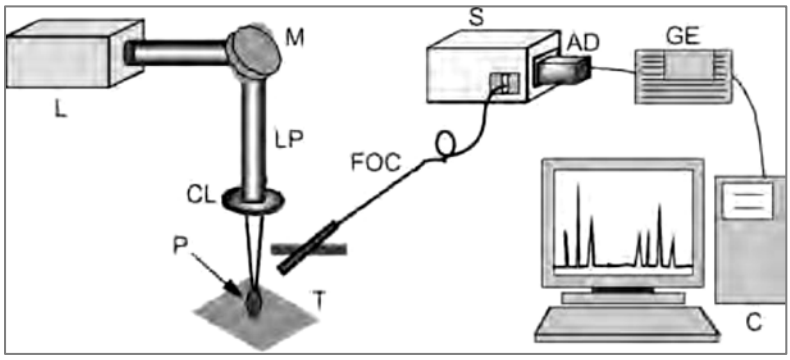

Figure 11. Typical LIBS system, showing the laser (L), mirror (M), laser pulse (LP), lens (CL), plasma (P), target (T), fiber optic cable (FOC), spectrograph (S), array detector (AD), gating electronics (GE), computer (C) [Miziolek 2006]

The characteristic wavelengths produced from relaxation processes of electrons are collected by a fiber optic cable, dispersed and focus by the spectrometer, and sensed by the detector [Miziolek 2006]. The most commonly used detectors for LIBS systems are the CCD and the iCCD. In general, the CCD behaves similar to a photo camera.

A computer is attached to the system and translates the signal obtained by creating a spectrum of discrete wavelengths versus the intensity of the emission signal. Each time the laser strikes the sample a spectra is created.

In order to achieve the best plasma optical emission several parameters can be optimized including: laser energy (%), laser repetition rate (Hz or shots/sec), gate delay (μs), spot size (μm), gate width (ms), gas flow (L/min), and whether a spot or laser ablation pattern will be use.

3.5.2 Principles and capabilities of ICP-OES

A generic ICP-OES system contains the following components: a spray chamber, the quartz torch, the focusing mirrors and lenses, the collection system (lens, mirror, or fiber optic), the detection system, which includes the spectrometer to filter or disperse the light and the detector, and the computer to translate and process the data.

Analysis by ICP-OES consists of introducing a small portion of the sample into the system and ionizing it in the plasma, where the light emitted is collected and sensed by the detector. The sample introduction procedure can be performed using an autosampler, for liquid samples or using a laser ablation system, for analysis of solid samples. The most essential part of this process is to introduce fine droplets or small particles in the ICP for ionization. For liquid samples, fine droplets can be created with

the aid of a pray chamber. There are different types of spray chambers, the most commonly known are: the concentric nebulizer, the cross-flow nebulizer, and the Babington nebulizer [Hou et al., 2000].

The cross-flow nebulizer set up will be used in this study. The set up consists of a flow of carrier gas perpendicular to the flow of liquid sample. The purpose of the carrier gas is to produce fine droplets that can enter the plasma and produce precise measurements.

For analysis of solid samples, a homogeneous stream of fine particles can be achieved by optimizing the parameters of the laser ablation system. The optimization of this laser ablation system varies with that of the optimization by LIBS in that the best ablation characteristics are seek, rather than plasma optical efficiency.

Once efficiency in sample introduction is achieved, a gas flow of helium (He) or argon (Ar) carries the droplets into the plasma. The plasma is generated inside a quartz torch. The torch consists of three concentric quartz tubes and a Tesla coil around the outer tube. In order to maintain the torch material cool, a carrier gas flow, usually Ar, is applied through the outer tube. Another flow of gas through the second concentric tube maintains the plasma cool and shapes the plasma.

The plasma starts in the presence of an Ar gas flow when a radio frequency (RF) power is applied to the Tesla coil. A spark initiates the plasma by interaction with Ar, and ionized Ar species are form, which in turn interact with other neutral Ar species. Therefore, the plasma is a combination of electrons, excited atomic species, and ionic species. The plasma is characterized as having different temperature areas, where the middle end of the plasma is usually chosen as the analytical area [Hou et al., 2000].

The light emitted by the species in the plasma is collected by mirrors that are located at an axial view or radial view of the plasma. The axial or radial views are used depending on the concentration of analytes in the solution. For trace elements the axial view is selected as it provides improved sensitivity over the radial view. This effect can be demonstrated by taking comparison measurements of both views with a standard solution.

The spectrometer in an ICP-OES system usually uses a grating to separate the wavelengths. Similar to the LIBS system the detector could be a CCD.

4 ANALYSIS OF GSR BY CMV-GC-MS

4.1 Experimental

4.1.1 Instrumentation

4.1.1.1 Analysis of GSR by CMV-GC-MS

The analysis of VOCs extracted with the CMV devices was performed with a gas chromatograph coupled to a single quadrupole mass spectrometer (GC-MS) equipped with a μECD detector. The GC-MS consists of an Agilent Technologies (Santa Clara, CA) GC system 7890A and a GC/MS Single Quad 5975C. The GC system is equipped with a Pneumatics Control Module (PCM), which allowed the coupling of the analytical column to both the single quadrupole and the μECD detector.

A Thermal Separation Probe (TSP) (Agilent Technologies, Santa Clara, CA) with a 4mm ID liner was used to thermally desorb the CMV devices into the GC-MS injector.

The analytical column used for this study was a 7.8 m DB-5ms Ultra Inert with 0.25 mm inner diameter, and a film thickness of 0.25 μm. The length of the column was reduced to 6.0 m towards the end of the project. The GC oven ramp temperature started at 40°C to 280°C beginning with a 0.5 min hold at 40°C and then increasing the temperature to 240°C at 15°C/min with a 5 min hold at 240°C. The temperature was then increased to 280°C at 30°C /min and held for 1 min at that temperature. The total time for the chromatographic separation was 21.16 min. The injector temperature was set at 180°C in split (split ratio 5:1) or splitless mode (5 min) with a column flow of 1.8 mL/min. The EI source was kept at 230°C, the transfer line to the mass spectrometer was set to 280°C and the quadrupoles were maintained at 150°C. The scan mass range was set

at 45-500 amu. The resolution of the mass analyzer is 0.1amu. The instrument was tuned before each experiment using the autotune feature as recommended by the manufacture.

The analytical performance of compounds expected to be present in GSR was evaluated. The targeted compounds consisted of: Nitroglycerin (NG), 2,4-dinitrotoluene (2,4-DNT), diphenylamine (DPA), and ethyl centralite (EC). The retention time and mass spectra profile for each compound was obtained from injecting a standard solution in the GC system.

Preliminary experiments for the detection of GSR with CMV were performed in a GC coupled to a triple quadrupole mass spectrometer (GC-QqQ). The GC-QqQ consisted of an Agilent Technologies (Santa Clara, CA) GC system 7890A and a GC/MS Triple Quad 7000B. The analytical column consisted of a 30 m HP-5ms Ultra Inert with 0.25 mm inner diameter, and a film thickness of 0.25 µm.

4.1.2 Reagent and standards

For optimization studies and calibration curves, single compounds standard solutions of nitroglycerine (NG) at 1000 ng μL^{-1} (AccuStandard, New Haven, CT), 2,4-dinitrotoluene (2,4-DNT) at 97% (Aldrich Chemical, Milwaukee, WI), diphenylamine (DPA), and ethyl centralite (EC) at 99% (Aldrich Chemical, Milwaukee, WI) were used to prepare stock solutions.

The stock solutions were prepared in-house to perform quantitative determinations. External calibration and standard addition methods were performed to characterize and quantify the organic compounds in the samples. Calibration curves for GC-MS analysis were prepared by direct liquid injection with the aid of an autosampler,

by direct spike in the CMV, and by headspace extraction with the CMV. The liquid injection and direct spike in the CMV consisted of spiking 1 μL of the standard solutions prepared in the range of 1.0 ppm (ng μL^{-1}) to 30 ppm, according to the expected amount for each compound in the samples. All the solutions were prepared in methanol as the solvent.

For headspace extraction analysis with the CMV, 1 μL of the standard solution was spiked inside a vial and the instrument signal was quantified against the direct spike in the CMV calibration curve. Therefore, the unit for amount detected on the spiked CMV is reported in ng, which was calculated by multiply the volume spiked (1 μL) times the concentration of standard solution analyzed (ng μL^{-1}).

4.1.3 Sample preparation

Minimum sample preparation was required to perform analysis by CMV-GC-MS. Swab samples were collected from the hands of a person using cotton applicators (100% cotton, Johnson&Johnson, Skillman, NJ). The cotton swab samples were placed inside 15 mL clear glass vials (Supelco, Bellefonte, PA) with phenolic screw caps and red rubber/PTFE septa to provide a proper seal for analysis of volatile organic compounds. The septa of the 15 mL glass vial caps were previously punctured to fit a CMV device after sample collection. After fitting the CMV, the device was covered with aluminum foil to avoid contamination of the sample and reduce humidity.

In the laboratory, a portable air sampling pump (Escort Elf Pump, Ocala, FL) operated at a constant flow of 1.50 L/min was used for headspace extraction. The CMV previously placed on the septum of the vials was attached to a tube connected to the

pump. After headspace extraction for 2 min, the volatile components adsorbed to the CMV, were analyzed by GC-MS with the aid of the thermal separation probe (TSP).

Analysis of the headspace from the cans with spent cartridges was performed with a CMV device previously conditioned in the laboratory. Conditioning the CMV consists of placing the CMV in an oven at 250°C for 30 min. A blank sample of the CMV is then analyzed in the GC-MS to assure that the device is clean.

4.1.4 Sample collection

An Institutional Review Board (IRB) application was filed and was continually renewed every year with the corresponding institution (Florida International University, Miami, FL). The IRB consent approval allows the collection of swab samples from the hands of police officers after shooting in a supervised training institution (Miami-Dade Public Safety Training Institute, Miami, FL), as well as swab samples from non-shooters at the university campus.

4.1.4.1 Sample collection from shooters

The GSR samples were collected from the hands of officers in an open range under typical shooting practice conditions. Personal information was not recorded at any stage of the sampling process.

The officers used three different types of ammunition during the range practice: pistol, rifle, and shotgun. The upper area of the right and left hands of a total of 43 officers were swabbed before and after shooting each type of ammunition. A total of 138 hand swab samples were collected from police officers for CMV-GC-MS analysis. Other

analyses by CMV-GC-MS consisted on headspace extraction of hands inside plastic boxes and headspace extraction near the hands of the shooter. A total of 35 samples were collected for that purpose.

Prior to field sampling, the CMV devices were conditioned in an oven at 250 °C for 2 hrs. Following conditioning, the CMVs were packed in sets of 4 in aluminum foil for transportation to the field.

The swabbing procedure was performed immediately after or within 30 min after shooting for all officers. The cotton swabs used for swabbing were previously moistened in deionized water (18 MΩ) and stored in 15 mL clear glass vials (Supelco, Bellefonte, PA) with phenolic screw caps and red rubber/PTFE septa to provide a proper seal for analysis of volatile organic compounds. The septa of the 15 mL glass vial caps were previously punctured to fit a CMV device after sample collection. After fitting the CMV, the device was covered with aluminum foil to avoid contamination of the sample and reduce the humidity.

4.1.4.2 Sample collection from non-shooters

The upper area of the right and left hands of a total of 6 non-shooters were swabbed. A total of 12 hand swab samples were collected from non-shooters for analysis by CMV-GC-MS. The swabbing procedure was performed in the same manner as described before (Section 3.1.4.1).

The discrimination and identification capabilities of CMV-GC-MS were evaluated between samples from shooters and non-shooters.

4.1.4.3 Sample collection from spent cartridges

Three ammunition types were considered for this study: pistol, rifle, and shotgun. The pistol ammunition was from American Eagle (Federal Cartridge Company) 9mm Luger, 124 GR. full metal jacket, or Winchester Ranger law enforcement ammunition 9mm Luger +P+, 127 GR. The rifle ammunition was from American Eagle (Federal Ammunition) .223 REM, 55 GR. full metal jacket boat-tail, or Winchester Ranger law enforcement ammunition .223 REM, 55 GR. ballistic silvertip. The shotgun ammunition was from Federal Premium law enforcement ammunition, 12 GA Buckshot, 2 ¾ inches.

A total of 45 spent cartridges were collected from each type of ammunition and were placed inside quart (~0.946 L) cans (All American Containers Inc., Miami, FL). Five (5) spent cartridges were placed inside each can. Also total of 15 spent cartridges from each type of ammunition were collected and stored individually inside nylon arson evidence bags (Grand River Products, LLC, Mt. Clemens, MI).

The GC-MS analysis was performed by headspace extraction with a CMV using the portable pump.

4.1.4.4 Blank cotton swabs

A total of 13 blank cotton swabs were treated using the same procedure for sample storage and analysis. These analyses allowed the determination of possible interference peaks near the retention time for the target compounds.

4.1.5 Data reduction and analysis

Data reduction and statistical analyses were performed with MSD ChemStation data analysis software (v E.02.01.1177 Agilent Technologies, Santa Clara, CA), and Microsoft Excel 2010 (v 14.0.7153.5000, Microsoft Corp., Redmond, WA).

4.2 Results and discussion

4.2.1 Development and optimization of CMV-GC-MS and μECD for the analysis of GSR

The optimal parameters for the detection of volatile organic compounds (VOCs) depend on the volatility of the compounds as expressed by the vapor pressure of each compound.

One of the challenges in the detection of VOCs from GSR is the amount of particles that can be collected from the hands of the shooter. The amount of particles will affect how much of the compounds are present in the headspace. Therefore, optimization of headspace extraction parameters is essential for the success of the extraction method.

The sample collection method selected for this work is practical and provides simple sample storage to perform inorganic and organic analyses. The glass vials used for sample storage and transportation provide an airtight seal to prevent organic compounds from escaping. Other advantages of using a small volume vial (15mL) for headspace extraction include: achieving fast equilibrium of the compounds with the headspace of the vial, and having a greater concentration of VOCs in a defined space, which can improve the extraction of all target compounds.

Earlier extraction procedures were performed by opening the cap of the vials and extracting the headspace. The major drawback of this procedure is possible sample loss

by opening the vial to the environment. Therefore, later extraction experiments were performed by fitting a CMV device through the cap septum of the vials and performing dynamic extraction by opening a second hole in the cap septum. The extraction procedure used here has the advantage of being able to perform static extraction prior to dynamic extraction, which provided higher recoveries for the extraction method.

In a headspace extraction technique there are two factors that can significantly influence the extraction and detection of compounds: the equilibrium time and the extraction time. These variables can be found experimentally using standard solutions of the compounds of interest.

A 1 µL mixture of the targeted compounds (NG, 2,4-DNT, DPA and EC) at 10 ppm was spiked into a 15 mL glass vial and extracted using the CMV device, as previously described.

The CMV headspace extraction parameters were optimized by creating a response curve at different equilibrium times (5, 10, 15, 20, 30 min) and extraction times (0.5, 1, 2, 3, 4, 5 min), at room temperature (20.0-21.0°C).

The selection of the optimization parameters was determined by the following criteria: high signal-to-noise ratio (SNR), precision (%RSD) and reproducibility, and selectivity.

The equilibrium time was selected on the basis of the most intense signal with the best SNR and best precision for the compounds of interest. Although the equilibrium curve seems to equilibrate within 5 min, the equilibrium time was selected at 20 min. Figure 12 shows the response curve for 10 ng of NG, 2,4-DNT, DPA, and EC spiked in a 15 mL vial. The amount extracted in the headspace of the vial does not increase

significantly over time. Also EC was not detected in the headspace at this concentration because of its low volatility. At the selected equilibrium time (20 min) the precision was below 12%RSD for all the detected compounds.

In the present study, the equilibrium time was optimized to generate headspace calibration curves and show the capability of CMV to provide quantitative analysis. In samples from hand swabs, the equilibrium time varies from the time the sample was collected until the dynamic headspace extraction is performed. Therefore, equilibrium experiments provide insight about the response of compounds over time but are not a parameter that was used for analysis of field samples.

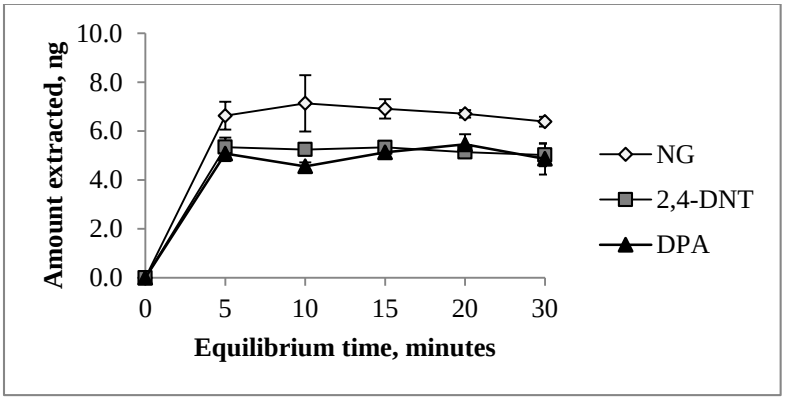

Figure 12. Equilibrium time experiment for the target compounds in a 15 mL vials using standard mixture solutions

The efficiency of the extraction method for the swab samples was tested by creating a response curve with equilibrium times (20 min, 2, 5, 10, 24, 30 hours) expected during the swab sample extraction process. The signal response obtained shows no sample loss even at 30 hours of equilibrium time. Therefore, the CMV is suitable for field

sampling and capable of retaining the compounds of interest during storage and until the samples can be analyzed.

The extraction time was selected using the most intense signal with the best SNR and best precision for the compounds of interest. A strong signal for DPA was obtained with most parameters, thus the selected extraction time of 2 min gave the best SNR for NG. Figure 13 shows the response curve for 10 ng of NG, 2,4-DNT, DPA, and EC spiked in a 15 mL vial. EC was not observed because of its low volatility. An extraction time of 1 min provided sufficient time to extract an equilibrium amount into the CMV. However, at the selected extraction time (2 min) the precision was below 24%RSD for all the compounds.

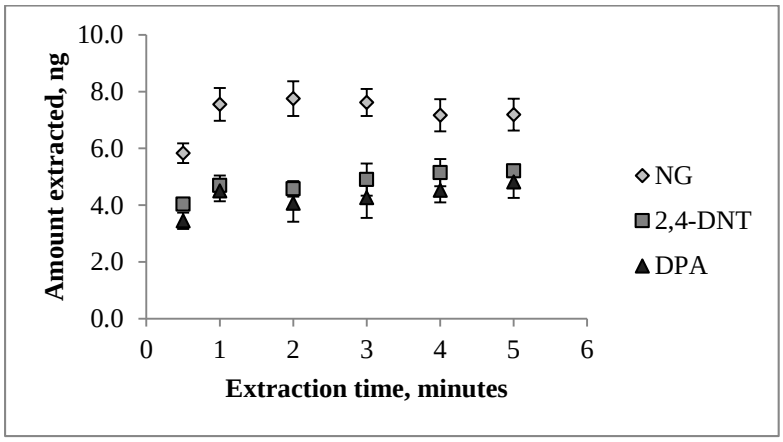

Figure 13. Extraction time experiment for the target compounds in a 15 mL vials using standard mixture solutions

For the extraction of compounds in the headspace of swab samples, extraction of volatiles is achieved first through static headspace extraction by the method described in the sample collection section (Section 4.1.4.1). Dynamic headspace sampling of the swab

samples is then performed by attaching the CMV device to a tube connected to a portable air sampling pump operated at a constant flow of 1.50 L/min. All headspace extractions for standard mixture solutions and samples were performed for 2 min.

4.2.1.1 Calibration strategy and the selection of the VOCs list for GSR detection

External calibration curves were created by CMV-GC-MS with standard solutions containing the compounds expected to be present in GSR samples.

The organic compounds (NG, 2,4-DNT, DPA, and EC) utilized in this study were initially selected from literature reports [Dalby et al., 2010; Joshi et al., 2011; Perr et al., 2005]. The selected compounds have been found to be present in the smokeless powders from the propellant [Dalby et al., 2010].

The retention time for each compound was determined by injecting a liquid standard solution in the GC. The retention time for NG, 2,4-DNT, DPA and EC were 6.54 min, 7.92 min, 8.65 min, and 10.44 min, respectively. The mass spectrum for each compound was used to determine the fragmentation of the compounds. The following ion peaks were used to confirm the presence of the target compounds at the expected retention time: NG (46.0 m/z and 76.0 m/z), 2,4-DNT (89.0 m/z, 119.0 m/z, 165.0 m/z, and 182.0 m/z), DPA (167.0 m/z, 168.0 m/z, 169.0 m/z, and 170.0 m/z), and EC (120.0 m/z, 148.0 m/z, and 268.0 m/z).

Calibration curves were generated by direct liquid injection at different concentrations (5.0 ppm-30 ppm) of DPA, NG, 2,4-DNT, and EC. Linearity of 0.952 or better was observed for all compounds by GC-MS.

Calibration curves were also generated with the optimized parameters by spiking 1 µL of mixture solutions at different concentrations (5.0 ppm-30 ppm) of DPA, NG, 2,4-DNT, and EC in 15 mL vials for headspace extraction or by direct injection on the CMV devices. The calibration curves generated by direct spike on CMV are similar to the calibration curves generated by headspace extraction with the CMV device. Good linearity was observed for all compounds for direct injection on the CMV (0.962 or better) and headspace extraction with the CMV (0.955 or better) by GC-MS.

The amount of volatiles extracted from the headspace of GSR samples was calculated using the headspace calibration curves. Calibration curves generated from µECD detection were used to calculate the amount of NG extracted from the headspace over cotton swabs. Similarly, the amount of DPA was calculated using the headspace calibration curves obtained with full-scan MS mode by ion extraction chromatogram.

Recovery studies for the direct injection of standards on the CMV yield 44%-97% efficiency for all the compounds, at 15 ng a point in the middle of the calibration curve. For the headspace extraction of compounds with the CMV the recovery yields 59%-87% extraction efficiency.

4.2.2 Figures of merit for CMV-GC-MS and µECD

The figures of merit for CMV extraction and analysis by GC-MS and µECD in split and splitless mode are summarized in Table 4 and 5, respectively. The compounds of interest show a linear response in the concentration range expected for GSR samples. In split mode, linearity of 0.952 or better was observed for all compounds by GC-MS.

The linearity for the same calibration by GC-µECD was 0.950 and 0.997 for NG and 2,4-DNT, respectively.

Linearity of 0.962 or better was observed for all compounds for direct injection on the CMV by GC-MS. The linearity for the same calibration by GC-µECD was 0.984 and 0.982 for NG and 2,4-DNT, respectively.

The headspace calibration curves had a linearity of 0.955 or better for NG, DPA, and 2,4-DNT by GC-MS. The linearity for the same calibration by GC-µECD was 0.982 and 0.993 for NG and 2,4-DNT, respectively.

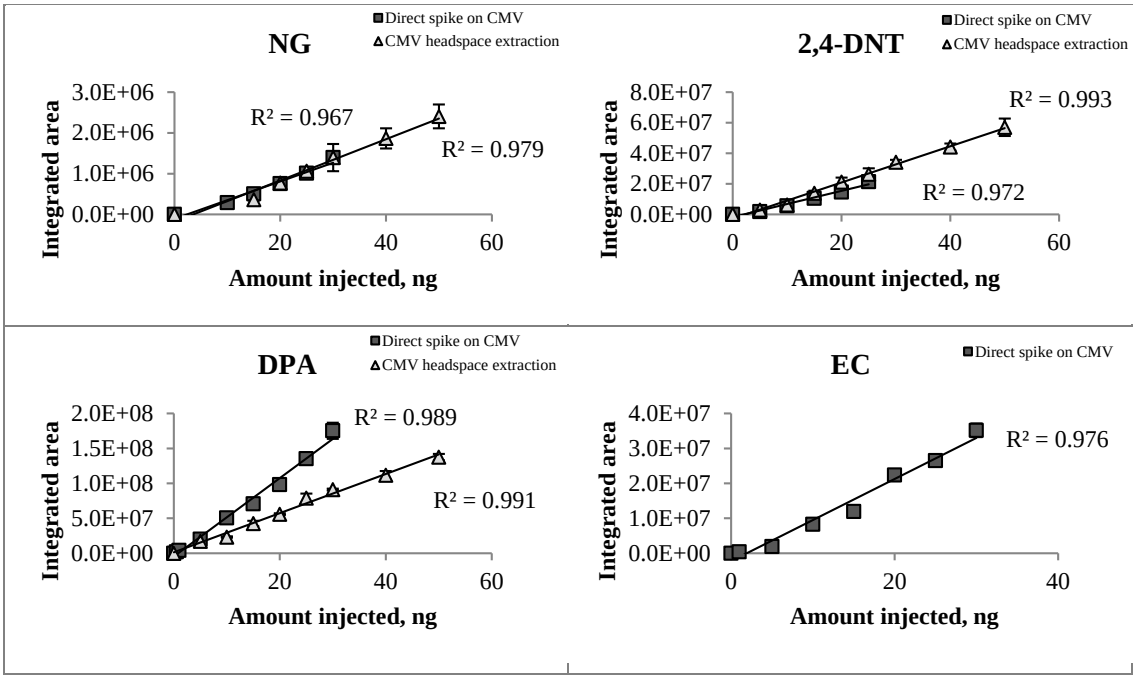

Figure 14. Calibration curves for direct spike on CMV and headspace extraction in splitless mode using mixture solutions of target compounds

In splitless mode, headspace extraction calibration curve for EC were generated in splitless mode and yield an R^2 value of 0.810. Ethyl centralite has low volatility and

higher concentrations are needed to detect the compound in the headspace. The linearity obtained in splitless mode was 0.979 or better for the other compounds. The calibration curves shown in Figure 14 were generated in splitless mode for the target compounds.

The method detection limits (MDL) for all compounds studied were determined for both direct spike analysis on CMV and headspace extraction. Detection limits for each compound was determined by GC-MS and μECD, by calculating the statistical $s_{y/x}$, which estimates random errors in the y values, and using equation 5.12 in the book [Miller 2005].

Table 4. Figures of merit for GC-MS and μECD in split mode for direct spike on the CMV and headspace extraction analysis with the CMV

		CMV-GC-MS			
		Direct spike		Headspace	
Compound	GSR Concentration range (ng)	MDL (ng)	%RSD	MDL (ng)	%RSD
NG	n.d.-3.9	7.0	17	2.1	4
2,4-DNT	n.d.	5.6	8	2.4	10
DPA	n.d.-2.0	1.3	11	3.0	8
EC	n.d.	2.0	32	36	8

		CMV-GC-μECD			
		Direct spike		Headspace	
Compound	GSR Concentration range (ng)	MDL (ng)	%RSD	MDL (ng)	%RSD
NG	n.d.-6.5	5.6	6	4.1	5
2,4-DNT	n.d.	3.1	2	1.9	11

In Table 4 and 5 a summary of figures of merit and expected GSR concentration range is shown for split and splitless mode, respectively. It is important to point out that the MDL reported here represents the minimum amount of analyte that can be spiked in

the vial to obtain a response. In addition, the amount of DPA detected in the samples was calculated using the extract ion chromatograms (eic) at 169m/z, the method shows detection limits of 3.3 ng in the splitless mode. Therefore, it can be stated that the MDLs are well below the concentrations detected in GSR samples.

Table 5. Figures of merit for GC-MS and µECD in splitless mode for direct spike on the CMV and headspace extraction analysis with the CMV

		CMV-GC-MS			
		Direct spike		Headspace	
Compound	GSR Concentration range (ng)	MDL (ng)	%RSD	MDL (ng)	%RSD
NG	n.d.-7.0	6.7	17	7.9	5
2,4-DNT	n.d.	5.3	4	4.3	15
DPA	n.d.-2.0	4.1	2	5.0	8
EC	n.d.	5.7	5	26	36

		CMV-GC-µECD			
		Direct spike		Headspace	
Compound	GSR Concentration range (ng)	MDL (ng)	%RSD	MDL (ng)	%RSD
NG	n.d.-12	4.5	19	7.2	32
2,4-DNT	n.d.	4.9	7	4.4	11

4.2.3 Results for the detection of volatiles on the hands of non-shooters

A blank study was performed by swabbing the right and left hands of 6 non-shooters. The cotton swab samples were placed inside the 15 mL vials and the same extraction procedure and analysis was followed as described for the GSR samples. The chromatograms showed no peak interferences at the retention times of the target compounds.

4.2.4 Results for the detection of volatiles on the hands of shooters

Preliminary results were performed with the collection of samples from 4 shooters by CMV-GC-QqQ. A total of 16 samples were collected from the left and right hands of the shooters, DPA was detected in 38% of the samples. Quantitation of DPA yield results in the range of 0.8-0.9 ng just above the method detection limit (0.7 ng).

Further studies were performed by GC-MS single quadrupole coupled to a μECD detector. Swab samples stored in the 15 mL vials with attached CMV devices were transported to the lab for headspace dynamic extraction and analysis by GC-MS. Criterion for the detection of target compounds consisted on the presence of the ion peaks at the expected retention times. The expected ion peaks for NG (46.0 m/z and 76.0 m/z), 2,4-DNT (165.0 m/z and 182.0 m/z), DPA (169.0 m/z, 168.0 m/z, 167.0 m/z), and EC (120.0 m/z, 148.0 m/z and 268.0 m/z) were used for identification. Confirmation of NG and 2,4-DNT was possible through simultaneous detection by μECD using the retention time for these compounds.

From the targeted compounds only NG and DPA were found to be present in the headspace of the GSR samples. Some samples contained both NG and DPA while in others only one of the compounds was present. An example of the μECD signal obtained from the headspace extraction of the swab samples is shown in Figure 15 [Tarifa et al., 2015]. Quantitation of NG was performed using the headspace calibration curve obtained with the μECD signal. Similarly, the amount of DPA in the samples was calculated using the GC-MS headspace calibration curve via the extract ion chromatogram.

A total of 28 officers participated in this study, 2.6-6.9 ng of NG were detected on the hands of shooters by GC-μECD. For DPA a range of 0.8-2.0 ng was detected in the

hands of shooters. An additional two shooters tested negative for the presence of both DPA and NG. The remainder of the samples produced a signal above the MDL. From the 40 samples taken from the right hand of shooters, NG was detected in 14 of the samples and DPA in 27 of the samples. However, the presence of NG or DPA was not detected on the right hands of 7 shooters.

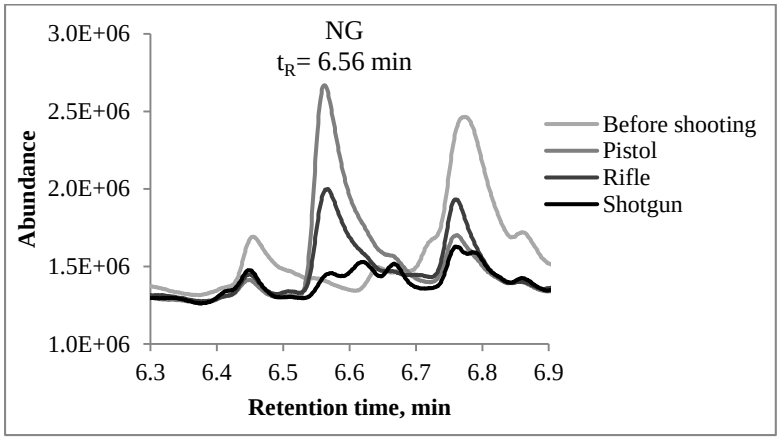

Figure 15. Detection of NG is the hands of officers after shooting pistol, rifle, and shotgun compared to a sample of the officer before shooting

4.2.5 Results for the detection of volatiles in spent cartridges

Analysis of spent cartridges from different types of ammunition (pistol, rifle and shotgun) with expected ng detection of the target VOCs by CMV-GC-MS can provide additional information regarding organic compounds that are present in the cartridge and that could be potentially transferred to the shooter's hands.

Spent cartridges were collected in quart cans (5 spent cartridges per can) or individually sealed in clear plastic bags that prevent diffusion of the sample. Table 6 shows the compounds present in the smokeless powders of the different types of

ammunition. From the compounds listed in Table 6, only NG, DPA, and EC were monitored in the samples.

Table 6. Compounds present in the smokeless powders of different types of ammunition used by shooters in the study

Compounds	Ammunition		
	Pistol and rifle		Shotgun
	Winchester® % by Weight	American Eagle® % by Weight	Federal Premium® % by Weight
Nitroglycerin (NG)	10-30 or 30-60	0-7	2-5
Dibutyl phthalate	1-5		
Ethyl centralite (EC)	3-7		
Ethyl acetate	0.5-1.5		
Rosin	1-5		
Diphenylamine (DPA)	0.5-1.5		
N-nitrosodiphenylamine	0.1-1		
Nitrocellulose	40-70	0.5-12	0.5-2

* Concentration range for all types and brands of ammunition (Section 4.1.4.3)

Headspace extraction of spent cartridges in the cans was performed 10 days after collection. The extraction procedure consisted of heating the quart cans at 60-70°C for 20 min followed by headspace extraction for 2 min. The presence of DPA was confirmed in the headspace of all spent cartridges. Nitroglycerine was detected in two pistol samples and the shotgun samples. The field blank quart cans did not contain any of the target compounds.

Quantitation of the signal was performed as described for GSR samples. The amount of DPA and NG detected show a clear distinction between shotgun cartridges and cartridges from pistol and rifle. The greatest amount of DPA and NG were detected in the headspace of shotgun spent cartridges at 89-370 ng and 5321-8170 ng, respectively. In

pistol spent cartridges, 2.2-26 ng of DPA and 2.9-11 ng of NG were detected, and an amount of 1.2-2.6 ng of DPA and 2.5-3.1 ng of NG was detected in rifle spent cartridges.

Headspace extraction of spent cartridges individually stored in plastic bags was performed at room temperature for 2 min extraction time. The presence of DPA was confirmed in the headspace of all spent cartridges. Nitroglycerine was detected in two pistol samples and the shotgun samples. The field blank quart cans did not contain any of the target compounds.

Quantitation of the signal was performed as described for GSR samples. As shown in Figure 16 NG and DPA were detected in all spent cartridges except for rifle cartridges. However, NG was only detected in two of the pistol cartridges and was found in the greatest amount for all spent cartridges. Similar to the results obtained from cartridges in quart cans, NG was most abundant in the shotgun spent cartridges.

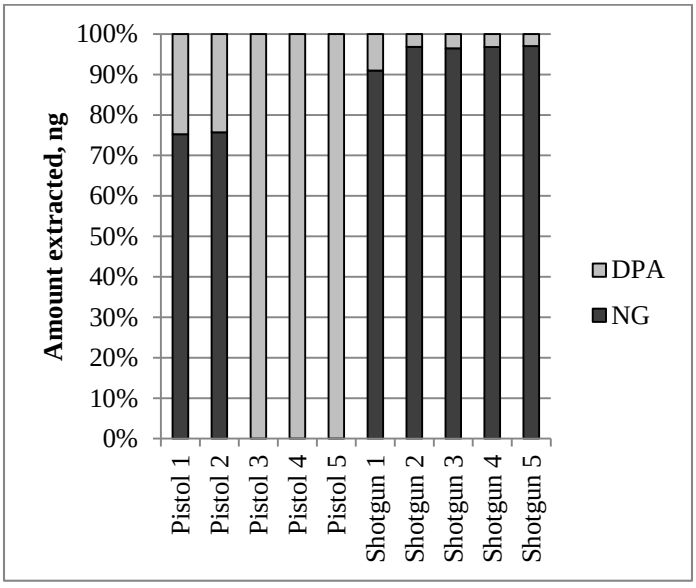

Figure 16. Detection of NG and DPA in spent cartridges individually sealed in plastic bags

The greatest amount of DPA and NG were detected in the headspace of shotgun spent cartridges at 0.9-3.6 ng and 28-94 ng, respectively. In pistol spent cartridges, 0.5-1.0 ng of DPA and 2.7-2.9 ng of NG were detected.

4.2.6 Evaluation of the significance of the organic analysis of GSR

The identification of GSR on the hands of a person in forensic laboratories is currently based on elemental detection and analysis [ASTM E1588-10]. As previously discussed the limitation of relying solely in this approach includes the possibility of finding particles with a composition similar to that of GSR. It has been found to be the case that similar elemental profile was detected on the hands of workers from different professions.

The method proposed in this works has the aim to provide unambiguous identification of GSR by detecting organic compounds commonly found in smokeless powders. In the previous sections the performance of CMV for the detection of volatile organic compounds in GSR was evaluated. The results demonstrated the suitability of CMV for the detection of compounds in the concentration range expected to be found in GSR samples.

However, identification of GSR should not be limited to organic analysis. A combination of techniques is beneficial for confirmation of the results. The advantages of using CMV are: the fast sampling time coupled to a fast GC-MS method (~20 min), sample preparation is not required, and the technique is nondestructive. Further improvements in sample collection can be established using aluminum stubs if analysis by SEM-EDX is desired.

4.3 Conclusions for the analysis of VOCs in GSR

A novel sampling device was used for the first time for the detection of volatile organic compounds in the headspace of samples collected from the hands of shooters. Qualitative and quantitative methods were developed for the analysis of GSR. The four compounds targeted in the analysis of GSR were: NG, DPA, 2,4-DNT, and EC. The four target compounds were selected because these were found with more frequency in the headspace of smokeless powders [Joshi et al., 2011].

A control group of non-shooters was recruited to determine the presence of target compounds on their hands. Studies performed with non-shooters yield negative results for the detection of organic compounds characteristic of GSR presence.

Preliminary results by GC-QqQ did not provide any improvement for the detection of target compounds on the hands of shooters. Instead, further enhancement of the extraction method consisted of attaching a CMV device to the septum of the glass vial. The recovery rates (up to 87%) obtained with CMV demonstrated the absorption capability of PDMS for the target compounds. Therefore, the static extraction process prior to dynamic extraction in the laboratory provided better response for the target compounds.

The use of GC-μECD provided increased sensitivity for the detection of NG in field samples. Identification of NG was confirmed through the retention time of the compound and by the presence of fragment ions in the fullscan mode by GC-MS. In addition, successful identification and quantitation of DPA provided further confirmation of the presence of trace amounts of smokeless powders on the hands of shooters.

The analysis of spent cartridges was performed to obtain information about the target compounds and additional compounds that could potentially transfer to the hands of a person after firearm discharge. The headspace composition of the spent cartridges was compared to the material safety data sheet (MSDS) provided by the ammunition manufacture. According to the MSDS information, all the ammunition used by shooters was double based, as demonstrated by the presence of nitroglycerin. The ammunition MSDS from Winchester® provided the most information for composition of the propellant. The propellant used for Winchester® ammunition contained three of the target compounds: NG, DPA, and EC. However, as previously discussed EC has very low volatility and was not detected in spent cartridges.

Therefore, the composition of the smokeless powder in the propellant is an indication of compounds that could be potentially transferred to the hands of a person. For the purpose of the present study, only two of the monitored compounds were detected on the hands of shooters, NG and DPA.

Finally the capability of CMV as a nondestructive sampling device is an attractive method for headspace analysis, which could compliment current analysis of GSR in forensic laboratories.

5 ANALYSIS OF GSR BY LIBS AND ICP-OES

5.1 Experimental

5.1.1 Instrumentation

5.1.1.1 Analysis of samples by LIBS

The LIBS analyses were conducted on a J200 system (Applied Spectra, Freemont, CA), equipped with a 266 nm ns-Nd:YAG laser. The LIBS system has a Flex sample chamber (Applied Spectra, Freemont, CA) to allow the flow of gas (i.e., Air, He, Ar) through the cell for plasma performance and washout of particles. The chamber has an automated translational sample stage (X,Y, Z) for quick focusing and positioning of the sample. Also, the LIBS system has two cameras, one to locate the sample inside the chamber and another one for focusing of the sample. The LIBS system was equipped with an Aurora 6-channel charge couple device (CCD) detector (190 nm to 1040 nm), with a resolution of <0.1 nm for UV to VIS and <0.12 nm for VIS to NIR. The light emitted was collected through a fiber optic cable located at a 45° angle from the ablation area. The acquisition software was updated with TruLIBS^{TM} emission database and Aurora data analysis (Axiom 2.1, Applied Spectra, CA).

The analytical performance for several emission lines of elements expected to be present in GSR was evaluated. The element menu consisted of the following elements: Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sb, Sr, Ti, and Zn. The emission lines for each element were obtained from the TruLIBS^{TM} database.

Preliminary experimentation for the project was conducted on a RT100HP system (Applied Spectra, Freemont, CA) equipped with a 1064 nm ns-Nd:YAG laser and a Czerny Turner spectrograph (Princeton Instruments, NJ) with an ICCD detector (Gen II,

Andor Technology, CT) and a dual grating turret (operated at 2400 grooves/mm). The LIBS system also has an automated sample stage that moves in the X, Y, Z coordinates.

5.1.1.2 Analysis of samples by ICP-OES

The ICP-OES analysis was conducted on the Optima 7300DV (PerkinElmer, Waltham, MA) integrated with an Echelle spectrometer and a segment charge coupled device (SCD) detector. Solution analysis was performed with a Scott spray chamber, equipped with a GemTip cross flow nebulizer. The nebulizer consists of a pneumatic, in which a high stream of gas flow perpendicular to the sample outlet to aid in the breakup of the liquid stream into a fine aerosol. The sample is introduced in the nebulizer by a sapphire capillary tip which is in direct contact with the solution during analysis.

Preliminary experiments with the ICP-OES were performed using a laser ablation system, Cetac LSX-500 (Cetac Technologies, Omaha, NE). The Cetac LSX-500 is equipped with a 266 nm ns-Nd:YAG laser. Mixing was accomplished in-line with the ablation cell, either before or after the cell. Sample introduction was conducted using Tygon tubing, formulation R-3603 with a 1/8” ID and ¼” outer diameter (Fisher Scientific, Saint-Gobain Performance Plastics, Valley Forge, PA), which was connected directly from the ablation chamber to the entrance of the ICP-OES where the torch is located.

The ICP-OES analysis was conducted to corroborate results obtained by LIBS because the output of the raw data is very similar for both systems. The same elemental menu was used for analysis by ICP-OES. The only differences between the LIBS and ICP-OES methods are the emission lines used for some of the elements and the analysis

of the samples was performed in solution by ICP-OES rather than by laser ablation as described in the preliminary results. The reason for selecting solution analysis for the samples was to obtain more sensitive results and to confirm the presence of the elements detected directly from the solution that was otherwise spiked on the Teflon for LIBS analysis.

5.1.2 Reagent and standards

For optimization studies and calibration curves, single element standard solutions of Cr, Cu, K, Ni, P, Pb, and Sb at 1000 ng μL^{-1} and Al, Ba, Ca, Fe, Mg, Mn, Na, S, Si, Sr, Ti, and Zn at 10000 ng μL^{-1} (Peak performance, CPI International, USA) were used to prepare stock solutions.

The stock solutions were prepared in-house to perform quantitative determinations. A Teflon disk (GAPI USA Inc., Clayton, OH) was used as a support to deposit the standard solutions for analysis by LIBS. External calibration and standard addition methods were performed to characterize and quantify elements in the samples. Calibration curves for LIBS were prepared by spiking 1 µL of the standard solutions prepared in the range of 0.100 ppm (ng μL^{-1}) to 300 ppm, according to the expected amount for each element in the samples. Calibration curves for ICP-OES solution analysis were prepared in the range of 0.0100 ppm to 100 ppm.

For LIBS analysis, the spiking of solution on the Teflon surface was expected to dry without any penetration into the material. Teflon was selected as the ideal support for the liquid solutions because it is a material of organic composition. The laser ablation parameters were optimized as to obtain the largest signal to background level and

minimize extensive removal of the Teflon material. In addition, the area ablated was similar to the drop size to assure removal of all the spiked elements. Therefore, the unit for amount detected is reported in ng, which was calculated by multiply the volume spiked (1 μL) times the concentration of standard solution analyzed (ng μL^{-1}).

5.1.3 Sample preparation

Minimum sample preparation was required to perform analysis by LIBS. The Teflon disk was previously ablated with a grid pattern (1.4 x 1.4 mm) for visibility when spiking the solution. Then a 1 μL of the solution with the elements of interest were spiked on each grid pattern and left to dry overnight.

For the samples obtained from the hands of a person, the cotton swabs (100% cotton, Johnson&Johnson, Skillman, NJ) were transferred into plastic test tubes (Fisherbrand, Waltham, MA), 12 x 75 mm polypropylene with blue snap cap and properly labeled. Liquid extractions were then performed by adding 250 μL or 300 μL of a 10% HNO_3 trace metal grade solution to each tube. The mixture was then vortexed for 1 min and centrifuged for 5 min.

In order to determine the elemental contribution from the cotton swabs, standard addition calibration curves were generated by ICP-OES. For the analysis of the standard solutions, cotton swabs were placed in plastic test tubes and 100 μL of standard solutions were spiked on the cotton. Liquid extractions were then performed by adding 300 μL of a 10% HNO_3 trace metal grade solution to each tube. The mixture was then vortexed for 1 min and centrifuged for 5 min. Following this process, an additional 1000 μL of 10% HNO_3 was added to all the tubes. The mixture was vortexed again for 1 min each and

centrifuged for 5 min. An aliquot of 1000 µL was transferred to 15 mL conical plastic tubes (Corning™ CentriStar™ Centrifuge Tubes, Corning, NY) with red caps used for solution analysis, and diluted to 5 mL with deionized water (18 MΩ).

5.1.4 Sample collection

An Institutional Review Board (IRB) application was filed and was continually renewed every year with the corresponding institution (Florida International University, Miami, FL). The IRB consent approval allows the collection of swab samples from the hands of police officers after shooting in a supervised training institution (Miami-Dade Public Safety Training Institute, Miami, FL), as well as swab samples from non-shooters at the university campus.

5.1.4.1 Sample collection from shooters

The GSR samples were collected from the hands of officers in an open range under typical shooting practice conditions. Personal information was not recorded at any stage of the sampling process.

The officers used three different types of ammunition during the range practice: pistol, rifle, and shotgun. The upper area of the right and left hands of a total of 43 officers were swabbed before and after shooting each type of ammunition. A total of 153 hand swab samples were collected from police officers for LIBS analysis and a total of 138 for ICP-OES.

The swabbing procedure was performed immediately after or within 30 min after shooting for all officers. The cotton swabs used for swabbing were previously moistened

in deionized water (18 MΩ) and stored in 15 mL clear glass vials (Supelco, Bellefonte, PA) with phenolic screw caps and red rubber/PTFE septa to provide a proper seal for analysis of volatile organic compounds. The septa of the 15 mL glass vial caps were previously punctured to fit a CMV device after sample collection. The sample preparation and analysis of organic compounds was described in Chapter 4.

5.1.4.2 Sample collection from non-shooters

The upper area of the right and left hands of a total of 16 non-shooters were swabbed. A total of 12 and 40 hand swab samples were collected from non-shooters for LIBS and ICP-OES analysis, respectively. The swabbing procedure was performed in the same manner as the hand swabbing for shooters.

The identification capability of LIBS was evaluated between samples from shooters and non-shooters. Analysis of the samples by ICP-OES was performed to confirm results obtained by LIBS and to evaluate identification between samples of both populations.

5.1.4.3 Sample collection from spent cartridges

Three ammunition types were considered for this study: pistol, rifle, and shotgun. The pistol ammunition was from American Eagle (Federal Cartridge Company) 9mm Luger, 124 GR. full metal jacket, or Winchester Ranger law enforcement ammunition 9mm Luger +P+, 127 GR. The rifle ammunition was from American Eagle (Federal Ammunition) .223 REM, 55 GR. full metal jacket boat-tail, or Winchester Ranger law

enforcement ammunition .223 REM, 55 GR. ballistic silvertip. The shotgun ammunition was from Federal Premium law enforcement ammunition, 12 GA Buckshot, 2 ¾ inches.

A total of 45 spent cartridges were collected from each type of ammunition and were placed inside quart (~0.946 L) cans (All American Containers Inc., Miami, FL). Five (5) spent cartridges were placed inside each can.

Elemental analysis was performed by swabbing the inside of the spent cartridges and then following the same liquid extraction procedure previously described. One (1) spent cartridge was analyzed per ammunition type by LIBS.

5.1.4.4 Blank cotton swabs

A total of 12 blank cotton swabs were treated using the same procedure for sample storage and analysis. These analyses allowed the determination of the elements detected by contribution of the cotton material.

5.1.5 Data reduction and analysis

Data reduction and statistical analyses were performed by either the use of Microsoft Excel 2010 (v 14.0.7153.5000, Microsoft Corp., Redmond, WA), Geopro (CETAC Technologies, v 1.0, NE), Aurora LIBS data analysis software (v 2.1, Applied Spectra, CA), and WinLab32 (PerkinElmer, Waltham, MA, USA).

5.2 Results and discussion

5.2.1 Development and optimization of LIBS for the elemental analysis of GSR

The composition of GSR is rather complex since it contains both organic and inorganic materials. Therefore, it is important to develop a sample preparation method that will homogenize the mixture to obtain representative chemical information.

Another challenge in the analysis of GSR is the potential for low number of particles characteristic (Ba, Pb, Sb) of GSR presence to be found on the hands of shooters. Since the sample is collected by swabbing methods the composition of the swabbing material contributes to the background or analytical noise.

Preliminary studies were performed with the RT100HP LIBS system to determine the best sample preparation strategies for GSR detection. The first attempt was to perform direct LIBS analysis on the collected swabs. Optimization experiments were performed as well as standard addition calibration curves. The major drawback with this method is that the analysis could be performed only on one side of the swab. Since the GSR particles were distributed along the cotton swab, direct analysis did not allow for bulk representation of chemical information.

A pre-concentration method would aid in the homogenization of the sample and also improve detection of the target elements. A method was developed which consisted of making pellets with the cotton swabs used for sample collection. The size of each pellet was 6 mm in diameter and ~2 mm in height. The advantage of this method for sample preparation is that only a small area is ablated and it allows for preservation of the remainder sample for future analysis, if desired.

The pellets were made with a benchtop pellet press (4350.L Carver Benchtop Pellet Press, Wabash, IN). The pelleting process consists of introducing the sample into a pellet sizer, achieving vacuum in the pellet sizer, and pressing the sample with a selected pressure. The time the vacuum was left on before pressing the sample and the selected pressure were optimized for this sample preparation method. Optimization was simple and the best conditions were selected according to the shape and robustness of the pellet. The pellet die used in this method was a stainless steel die to create pellets with 6 mm in diameter. The condition of choice was leaving the vacuum on for 5 min before pelleting the sample up to a pressure of 3000 psi. Figure 17 shows a sample of a pellet made using the optimized conditions.

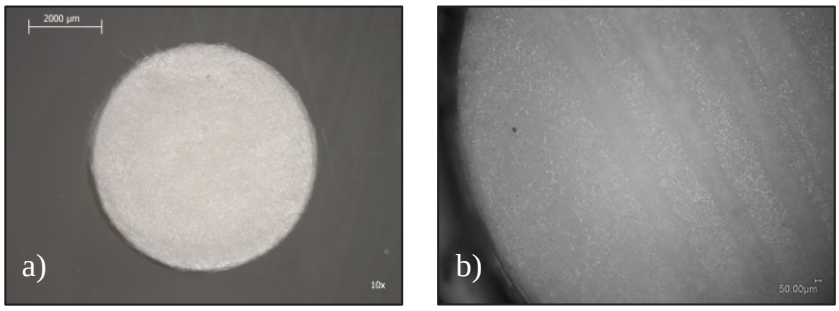

Figure 17. Sample from a) a cotton swab pressed into a pellet using optimized conditions (Leica Microscope, USA) and b) the raster lines produced by the CETAC laser on the cotton pellet (Keyence Microscope, USA)

The analysis of the cotton pellets was performed by LIBS (RT100HP). The analysis by RT100HP was conducted with previously optimized parameters (10x10 grid, 1.2 mm in dimensions, 2 accumulated shots per grid point, 90% laser energy, 1 µs gate delay, 3 Hz). Standard addition calibration curves were constructed in the range of 10-160 µg in the bulk cotton swab. The calibration curves show good linearity (0.980 to

0.869) for the elemental menu consisting of: Al, Ba, Cu, Ni, Pb, Sb, Sr, and Zn. The method detection limits ranged from 2-35 µg (2000-35000 ng), depending on the element.

The drawback of making cotton pellets is that only a small portion of the spiked elements is ablated and detection is above the range expected in field samples. The analysis of the cotton pellets from GSR samples showed signals above the detection limit for Ba, and no detection of Pb and Sb in any of the samples. One of the disadvantages of the pellets preparation method for GSR analysis is that dilution of the elements occurs along the volume of the pellet reducing the detection capability. Moreover, the stainless steel die used for making the pellets may contaminate the samples which lowered the possibility of identifying other elements for detection of GSR.

A sample preparation method was proposed to account for the following considerations: pre-concentration of the sample in order to detect the lowest amount of elements possible, allow homogenization of the sample, and reduce the background contribution from the spectra. The new method consisted on extracting the elements present in GSR from the cotton swabs used for sampling with the minimal amount of solvent and to spike an aliquot of the sample onto a surface that will create minimal background contribution. A Teflon substrate is ideal to use as a surface because it is made of a polymer (polytetrafluoroethylene), thus it is not expected to find any signal from the target elements.

The optimal parameters for LIBS measurements depend on the general purpose of the analysis but more essentially on the optical emission of the plasma. The use of an inert gas such as argon (Ar) aids in the formation of the plasma and creates a clean

environment for the plasma to avoid interferences from species present in ambient air. The plasma changes shapes depending on the gas used [Miziolek 2006].

The optimization of the LIBS parameters were carried out using helium (He) and argon (Ar) at different gas flows. The optimized parameters using Ar gas provided higher signal intensity for all the target elements. In addition, the gas flow was optimized to obtain better plasma formation and greater signal acquisition. Figure 18 shows that the optimal flow rate was achieved at 0.60 L/min. At the optimal flow rate, the SNR is largest and the %RSD is below 10%.

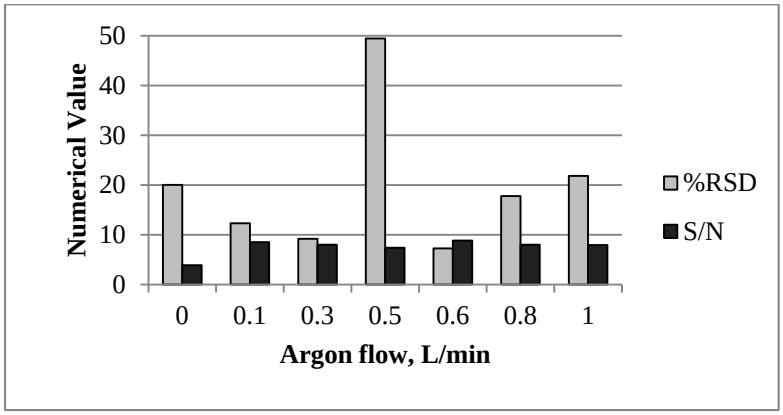

Figure 18. Ar gas flow rate optimization results for 100 ng of Sb (I) 259.8 nm

The J200 LIBS system parameters were optimized to ablate all the area of a thin solution layer created by the spiked sample over the Teflon surface. A grid of 12x12 (1.6 mm grid size, 0.15 mm grid points separation) was created to cover the entire sample area (~1 mm). Optimization of the laser and detector parameters for LIBS consisted of the evaluation of flash lamp energies (40-100 %, increments of 10%), laser shot repetition rates (1 Hz, 2 Hz, 4 Hz, 5 Hz, 8 Hz, 10 Hz), gate delays (0.1-3 μs), and spot size (45-200

μm). The number of shots was kept constant at 125 shots for all parameters during the optimization.

The selection of the optimization parameters was determined by the following criteria: high signal-to-noise ratio (SNR), precision (%RSD) and reproducibility, selectivity, and minimal removal of the Teflon material.

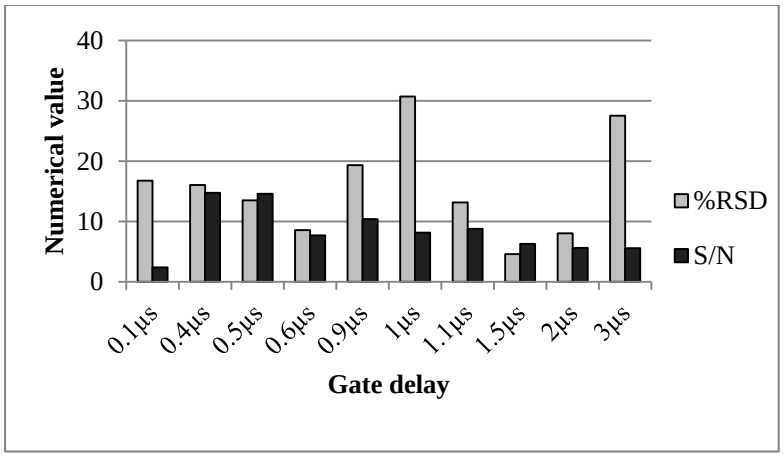

Figure 19. Gate delay optimization results for 100 ng Sb (I) 259.8 nm spiked on a Teflon surface

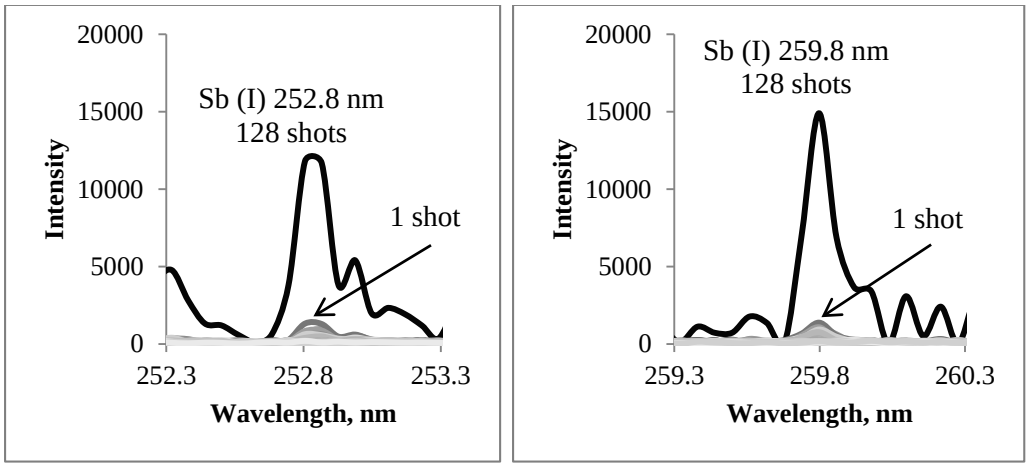

Figure 20. Spectra overlay for LIBS showing Sb (I) 259.8 nm and Sb (I) 252.8 nm after 1 shot and after 128 accumulated shots

The gate delay was selected based on the most intense signal with the best SNR and lowest precision for the principal target elements, Ba, Pb, and Sb. A strong signal for Ba was obtained with most parameters, thus the selected gate delay of 0.5 µs gave the best SNR for Pb and Sb. Figure 19 shows an example for gate delay optimization results for 100 ng of Sb (I) 259.8 nm spiked on a Teflon surface. At the selected gate delay the precision was below 15% RSD for all elements used in the optimization.

In LIBS experiments, the SNR can be improved by accumulating the signal from multiple shots. Figure 20 shows the improvement of the Sb emission lines when the shots are accumulated. The ablation parameters were optimized for a grid that covered the entire surface area where the sample was spiked on the Teflon. The parameters were chosen to obtain the best SNR by ablating the least amount of the Teflon surface and to remove most of the spiked solution. A total of 128 shots at a laser frequency of 10Hz and a speed rate of 0.85 µm sec^{-1} provided the desired results. The same criteria were followed to select the laser energy at 70% (~13 mJ). Table 7 summarizes the optimization parameters for LIBS (J200).

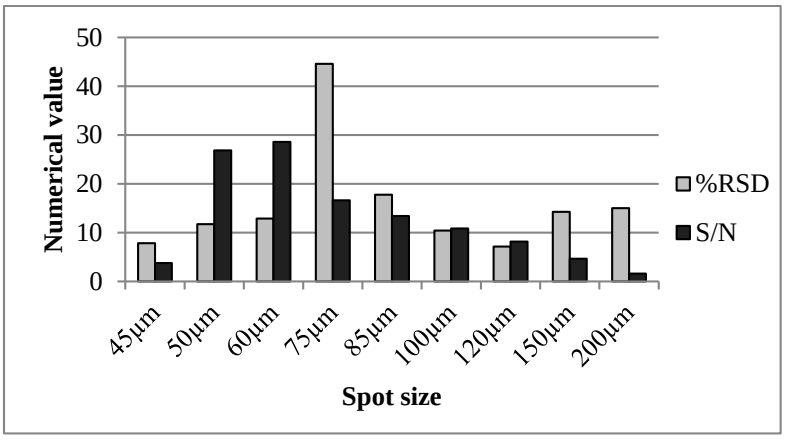

Figure 21. Spot size optimization for 100 ng of Sb spiked on the Teflon surface

An important parameter for the optimization of the method was the spot size. The spot size was optimized to avoid an excessive background contribution from the Teflon from overlapping laser shots. The optimized spot size was 60 µm, where a precision of 21% RSD or better was achieved for the elements used except for Ba, which had a precision of 30% RSD. Figure 21 shows an example for the spot size optimization results for 100 ng of Sb (I) 259.9 nm, spiked on a Teflon surface.

Table 7. Optimized parameters for the analysis of GSR by LIBS

Parameters	**LIBS (J200)**
Laser	266 nm Nd:YAG
Flash lamp voltage	70% (~13 mJ)
Gate delay	0.5 µs
Gate width	1.1 ms (fixed)
Spot size	60 µm
Repetition rate	10 Hz
Scan rate	0.85 µm sec^{-1}
Number of shots	128 (accumulated shots)
Ablation mode	Grid (8x8 line pattern)
Sampling time	12.50 sec
Sampling area	1.05 x 1.05 mm
Element menu	Ba, Pb, Sb

5.2.1.1 Calibration strategy and selection of the element list for LIBS

External calibration curves were created by LIBS with standard solutions containing the elements expected to be present in GSR samples.

The element menu (Al, Ba, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, Pb, S, Sb, Si, Sn, Sr, Ti, Zn, Zr) utilized in this study was initially selected from literature reports [Dalby et al., 2010 Review; ASTM E1588-10]. The selected elements have been found to be present in parts of the ammunition such as the primer mixture, the casing, and

the bullet [Dalby et al., 2010 Review]. Seven elements: Co, Li, P, S, Si, Sn, and Zr were discarded from the elemental menu either because these were not detected in GSR samples from preliminary results, were not present at the working concentrations in the method, or because of signal interferences with other important elements (i.e. Pb, Sb, Sr).

The emission lines for each element were carefully selected in order to use lines that corresponded to the element and to avoid potential signal interferences among the elements. One emission line was used for quantitative analysis; however, 2 to 3 emission lines were monitored for qualitative analysis to confirm the presence of the elements in the sample. All the emission lines monitored for each element are summarized in Table 8 (Section 5.2.3).

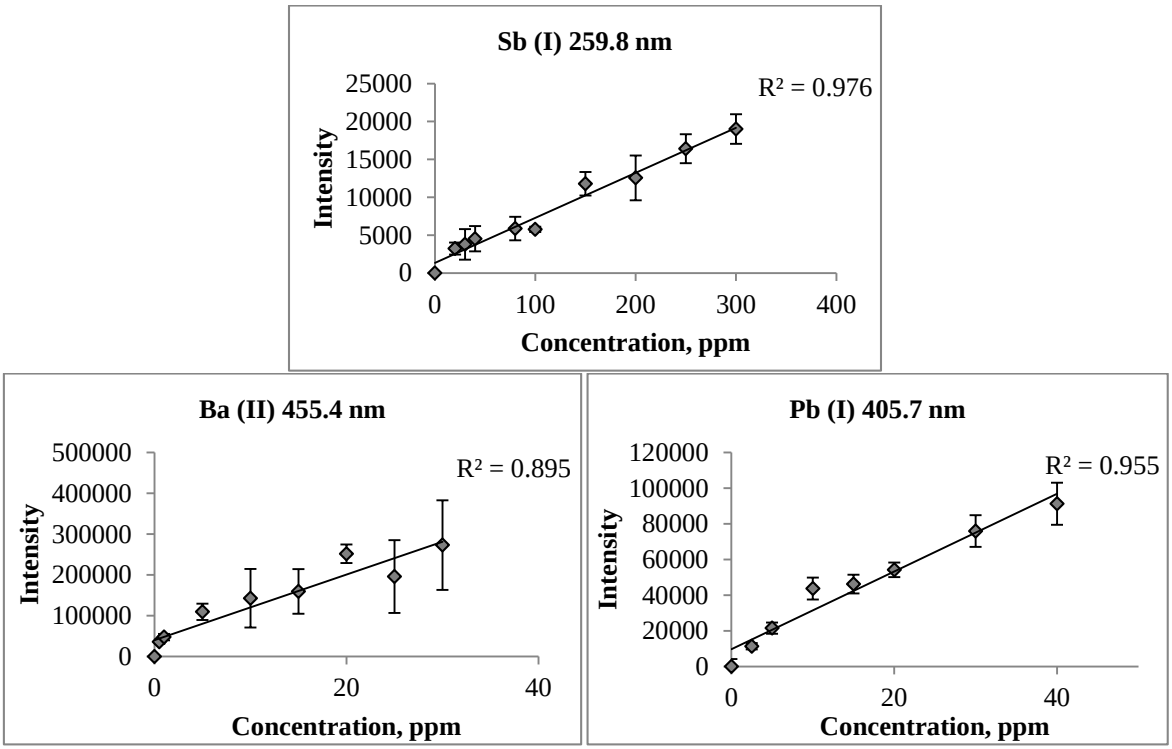

Figure 22. Calibration curves for Ba (II) 455.4 nm, Pb (I) 405.7 nm, and Sb (I) 259.8 nm by spiking 1µL of the standard mixture on the surface of Teflon

Calibration curves by LIBS were also generated with mixture solutions of the target elements at different concentrations (0.50-30 ppm). For Sb, the concentrations tested ranged from 20-300 ppm and for Pb 5-40 ppm to account for the higher MDL for these elements. Linearity of 0.887 or better was achieved for most elements of interest except for Fe which showed an R^2 of 0.843. Figure 22 shows the calibration curves obtain by LIBS for Ba, Pb, and Sb.

Other elements were tested for potential use as internal standards including Li, Y, Sc, and In. However, many signal interferences were found from the emission lines compared to the emission lines from the target elements.

5.2.2 Development and optimization of ICP-OES for the elemental analysis of GSR

The concentration of elements in GSR from the hands of shooters was expected to be very low and confirmation with a more sensitive technique was desired. The selection of ICP-OES for confirmation of LIBS results allows for direct comparison of several emission lines and also provides more sensitive results for important elements such as, Sb and Pb.

Preliminary studies were performed with a sample preparation method which consisted of making pellets from cotton swabs, as previously mentioned. The size of each cotton pellet was 6 mm in diameter and ~2 mm in height (Figure 17, Section 5.2.1). The cotton pellets were analyzed using a CETAC laser system coupled to the ICP-OES. Coupling of a laser system to the ICP-OES is very simple and is commonly done for glass analysis.

The CETAC laser system is different to LIBS in that the mass removed from the sample by the laser is transferred to the ICP and the signal detected is from the emission of the ICP. Therefore, the quality of the signal obtained depends heavily on the ablation efficiency to produce small particles (µm in size), which are homogeneous in order to create a flat transient signal.

Optimization of the laser parameters was performed prior to the analysis of GSR samples. The CETAC system parameters were optimized to ablate a portion of the cotton pellets and allowed to perform several replicates on the same pellet. A raster line was created with two different lengths, 500 µm and 700 µm, at different ablation rates (10-35 µm/sec). Optimization of the laser consisted on the evaluation of flash lamp energies (10-100 %, increments of 10%, and 30-50 %, increments of 5%), laser shot repetition rates (1 Hz, 5 Hz, 10 Hz), and spot size (100 µm, 200 µm, 250 µm). The number of shots varied depending on the repetition rate used.

The selection of the optimization parameters was determined by the following criteria: high signal-to-noise ratio (SNR), precision (%RSD) and reproducibility, selectivity, and shape of the transient signal.

The laser parameters that produced the best transient signal with a precision of 6% RSD for Sb (I) 206.8 nm were: 40% laser energy, 5 Hz, and a spot size of 200 µm. An ablation rate of 25 µm/seconds was selected for the required acquisition time with a raster length of 700 µm. Ideally, a base signal is obtained for 20 seconds with the laser off, 20-40 seconds with the laser on, and an additional 10-20 seconds with the laser off again. The raster length and laser conditions are optimized as to obtain the best transient signal with a relatively short analysis time (~80 seconds).

As previously discussed the sample preparation method used in the analysis by CETAC-ICP-OES provided low signal or no detection for the elements of interest (Sb, Pb, Ba) in GSR collected from spent cartridges. The calibration curves were constructed in the range of 1-80 µg and the detection limits for all elements were in the range of 2-14 µg, depending on the element. The amount of each element (Ba, Pb, Sb) detected in the spent cartridges was at or below the method detection limit (MDL).

As a result, a new sample preparation method was proposed as described in Section 5.2.1. The extraction of elements from the cotton swabs was carried out following a method described by Koons et al. (1988) for GSR analysis by flame atomic absorption spectrophotometry (FAAS). The extraction method was modified to accommodate analytical strategies for LIBS analysis. Similarly, analysis by ICP-OES was performed in solution to complement the LIBS analysis and to provide confirmation for the detected elements.

The extraction method consisted of adding 300 µL of 10% HNO_3 to the cotton swabs, vortexing the mixture for 1 min and centrifuging for 5 min. At this point a 1 µL aliquot was spiked on the surface of a Teflon disk for LIBS analysis. Three (3) to four (4) replicates were performed for LIBS analysis. The remainder of the solution was further diluted with 1000 µL of the 10% HNO_3. The mixture was vortexed again for 1 min and centrifuged for 5 min. Subsequently, a 1000 µL aliquot was extracted and transferred to plastic conical tubes for ICP-OES solution analysis. The sample was then diluted at a final volume of 5 mL (5000 µL) with deionized water (18 MΩ).

The ICP-OES was operated under typical parameters for the analysis of solutions. The radio frequency (RF) power was set at 1250 RF, 15 L/min plasma flow, 0.5 L/min

auxiliary gas, 0.5 L/min nebulizer glass, and 1.5 L/min pump speed. Washings with 1% HNO_3 were performed in between samples. The warm-up of the instrument and daily performance was done as suggested by the manufacturer. Data analysis for the samples was performed with the system software in order to obtain peak area, standard deviation, and precision.

5.2.2.1 Calibration strategy and selection of the element list for ICP-OES

External and standard addition calibration curves were created by ICP-OES with standard solutions containing the elements expected to be present in GSR samples. The standard addition calibration curves were used for quantitative analysis of GSR samples.

The element menu (Al, Ba, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, Pb, S, Sb, Si, Sn, Sr, Ti, Zn, Zr) utilized in the study was initially selected from literature reports [Dalby et al., 2010; ASTM E1588-10]. The selected elements have been found to be present in parts of the ammunition such as the primer mixture, the casing, and the bullet [Dalby et al., 2010]. Seven elements: Co, Li, Sn, and Zr were discarded from the elemental menu either because these were not detected in GSR samples from preliminary results, were not present at the working concentrations in the method, or because of signal interferences with other important elements (i.e. Pb, Sb, Sr).

The emission lines for each element were carefully selected to use lines that were particular to the element and to avoid potential signal interferences among the elements. The selected emission lines for quantitative analysis of GSR samples are reported in Table 8. One emission line was used for quantitative analysis and 2 to 3 emission lines

were monitored for qualitative analysis to confirm the presence of the elements in the sample.

For simplicity of visualization of the results, unless otherwise stated the quantitative results presented throughout this paper were performed using the quantifier emission line.

Table 8. Elemental menu reporting the quantifier emission lines and the qualifier (Q1 and Q2) emission lines for analysis by LIBS and ICP-OES

	LIBS			ICP-OES		
Element	Quantifier emission (nm)	Q1 (nm)	Q2 (nm)	Quantifier emission (nm)	Q1 (nm)	Q2 (nm)
Al	396.1	394.4	309.2	396.1	394.4	309.2
Ba	455.4	614.1	493.4	455.4	493.4	233.5
Ca	393.3	396.8	422.6	317.9	422.6	393.3
Cr	428.9	425.4	283.5	205.5	267.7	283.5
Cu	324.7	327.3	224.7	324.7	327.3	224.7
Fe	274.6	274.9	275.5	238.2	239.5	234.3
K	766.4	769.8	404.7	766.4	404.7	-
Mg	280.2	285.2	518.3	279.5	280.2	285.2
Mn	259.3	260.5	294.9	257.6	259.3	260.5
Na	588.9	589.5	330.2	588.9	589.5	-
Ni	361.9	341.4	352.4	231.6	232.0	341.4
P	-	-	-	213.6	178.2	214.9
Pb	405.7	368.3	363.9	220.3	217.0	405.7
S	-	-	-	181.9	180.6	182.5
Sb	259.8	252.8	323.2	206.8	217.5	231.1
Si	-	-	-	288.1	212.4	251.6
Sr	407.7	421.5	460.7	407.7	421.5	232.2
Ti	334.9	334.1	336.1	336.1	334.9	334.9
Zn	481.0	334.5	330.2	206.2	202.5	213.8

After analysis by LIBS, the presence of elements in the swab samples were confirmed by solution analysis with an ICP-OES by following the extraction method with the addition of 1000 µL of 10% HNO_3 as described in Section 5.2.2. Calibration curves by solution ICP-OES were generated with mixture solutions of the target elements at

different concentrations (0.250 ppm-15.0 ppm). Good linearity of 0.999 or better was achieved for all elements of interest. Recovery studies for the proposed sample preparation method yield 76%-100% extraction efficiency with the described method.

5.2.3 Figures of merit for LIBS and ICP-OES

All the results reported in this and the following sections were obtained from analysis by the J200 LIBS system and solution ICP-OES.

The figures of merit for LIBS (J200) and ICP-OES are summarized in Table 9. The emission lines for the target elements show a linear response in the concentration range expected for GSR samples. The R2 values ranged from 0.999 to 0.887 for LIBS (except, the lowest R^2 value for Ba was 0.859 and for Fe was 0.843), and 0.999 to 0.994 for ICP-OES (except for K (I) 404.7 nm was 0.967). The %RSD reported in Table X represents the precision of the measurement at a concentration in the middle of the calibration curve.

For LIBS, some elements have higher method detection limits than the expected concentration found in the samples or the method detection limits is at the concentration found in the samples (Table 9). The MDL was calculated using Equation 1 (Section 2.1.2) to find the response and using the response of a sample concentration at the MQL to calculate the MDL concentration as follow:

$$MDL\ (ppm) = \frac{Response_{concentration\ 1}}{Concentration_1} \times Response_{MDL}$$

The instrument response for concentration 1 in the relationship is obtained using the data analysis software. To confirm the results obtained with this method, a concentration near to that of the expected MDL was analyzed.

Table 9. Figures of merit for LIBS (J200) and ICP-OES (solution) for the quantifier emission lines and comparison with expected concentration of GSR on the hands of shooters

		LIBS			ICP-OES		
Element	GSR Concentration range (ppm)	MDL (ppm)	MQL (ppm)	%RSD	MDL (ppm)	MQL (ppm)	%RSD
Al	0.3-8.1	0.5	3.8	14	0.04	0.1	2
Ba	0.1-24	0.3	4.7	35	0.005	0.02	1
Ca	15-104	0.2	1.0	28	3.3	11	1
Cr	n.d.	2.1	7.3	28	0.01	0.02	1
Cu	0.1-15	1.0	6.7	15	0.002	0.01	1
Fe	0.7-13	9.1	42	19	0.03	0.1	1
K	15-392	0.7	4.0	30	0.1	0.3	2
Mg	1.6-12	0.2	1.5	25	1.0	3.2	1
Mn	n.d.	2.9	14	27	0.01	0.03	1
Na	17-368	0.2	0.5	18	14	45	2
Ni	0.1-3.1	3.1	10	19	0.004	0.01	1
P	n.d.	-	-	-	0.9	3.1	1
Pb	0.4-8.5	1.9	10	10	0.01	0.05	1
S	2.6-26	-	-	-	0.2	0.4	1
Sb	0.3-4.5	20	152	5	0.01	0.05	1
Si	n.d.	15	62	58	1.7	5.6	1
Sr	0.1-0.2	0.1	0.4	23	0.005	0.02	3
Ti	n.d.	1.4	6.5	32	0.01	0.03	1
Zn	0.1-11	1.7	6.0	21	0.002	0.01	1

As shown in Table 9 for ICP-OES results, MDL is lower for those elements that are not present in the cotton swabs, whereas if the element is present in the cotton swab the MDL was calculated as three times the signal for that element. Method detection limits for ICP-OES were low enough to detect characteristic elements (Ba, Pb, Sb) in the GSR samples and provided good confirmatory results. In addition, external calibration

curves were generated to determine the instrument limit of detection for the elements of interest.

5.2.4 Results for the detection of elements in blank cotton swabs

The contribution of elements already present in blank cotton swabs was evaluated by analyzing a total of 24 cotton swabs. The developed extraction procedure previously described was followed for this analysis. For all the samples 3-4 replicates were analyzed by LIBS and 5 replicates by solution ICP-OES.

The elements detected in blank cotton swabs by LIBS were: Al, Ca, Mg, and Na. Calcium and Na were present in almost all of the samples. Results were confirmed and additional elements were detected by solution ICP-OES in the blank cotton swabs: Ba, Fe, Mn, P, Si, and Sr.

Table 10. Example of the concentration range for elements expected to be present in blank cotton swabs analyzed by LIBS and ICP-OES

Elements	LIBS Concentration (ppm)	ICP-OES Concentration (ppm)
Al	n.d.-6.2	0.2-0.6
Ba	n.d.	n.d.-0.05
Ca	14-53	8.0-38
Fe	n.d.	n.d.-0.4
Mg	n.d.-20	0.6-9.0
Mn	n.d.	0.02-0.1
Na	14-50	12-97
P	n.d.	2.2-12
Si	n.d.	0.8-18
Sr	n.d.	n.d.-0.1

Quantitative analysis of elements present in cotton swabs was performed with LIBS and solution ICP-OES. Table 10 shows an example of 10 cotton swabs that were

analyzed by LIBS and ICP-OES. The calculated concentrations for elements using both techniques agree within certain degree. More variability is observed for Al, but it is suspected that there was some overlap of Al (I) 396.1 with Ca (II) 396.8 in the LIBS spectra. Also, small amounts of Ba were detected in 9 of the samples, however as reported in Table 10 the concentrations found in the cotton swabs are well below the concentrations found in GSR samples.

5.2.5 Results for the detection of elements on the hands of non-shooters

5.2.5.1 Results from LIBS analysis

Cotton swabs from a total of 6 non-shooters were collected from the right and left upper areas of the hands. A total of 12 hand swab samples were collected from non-shooters for LIBS (J200) analysis. Two (2) of the 6 non-shooters were spectators in the vicinity of the discharge area.

The elements detected in the hands of non-shooters included: Al, Ca, K, Mg, Na, and Sr. Figure 23 shows the distribution of elements detected on the hands of non-shooters. Copper (Cu) was detected in one replicate from the left hand of one non-shooter, but the concentration was below the MQL. Also, Sr was detected below the MQL for all non-shooters, except for the right hands of one non-shooter at a concentration of 0.7ppm.

From the elements detected on the hands of non-shooters, 4 were detected on the blank cotton swabs (Al, Ca, Mg, and Na). Quantitative analysis results demonstrated that concentrations for these elements were in the range of those found in blank cotton swabs.

Another element detected on the hands of all non-shooters was K with concentrations above the MQL for 5 of the non-shooters.

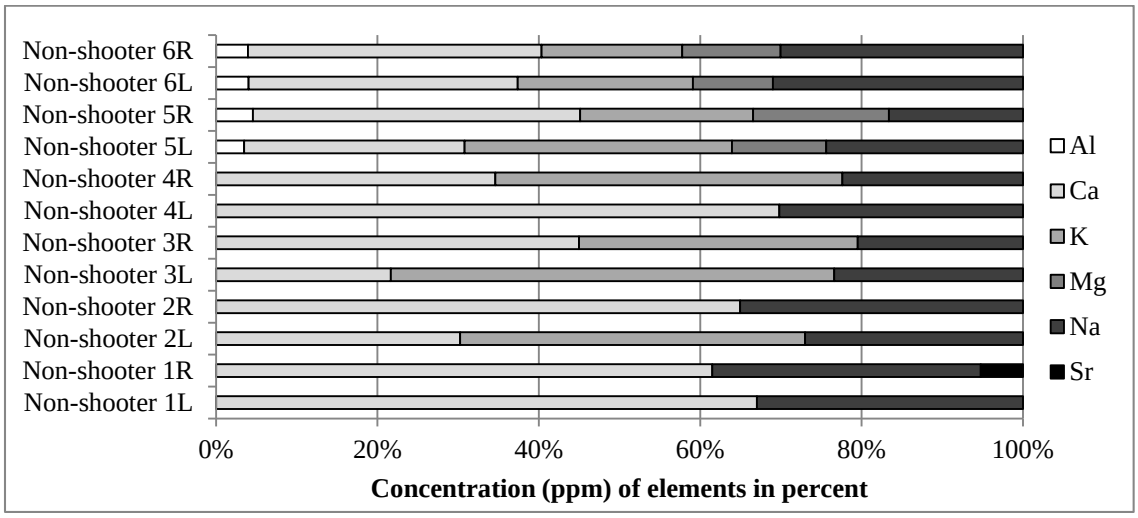

Figure 23. Distribution and concentrations represented in percentage for elements detected on the left (L) and right (R) hands of 6 non-shooters by LIBS

5.2.5.2 Results from ICP-OES analysis

Cotton swabs from a total of 16 non-shooters were collected from the right and left upper areas of the hands. A total of 40 hand swab samples were collected from non-shooters for solution ICP-OES analysis. These samples included the ones analyzed by LIBS. In addition, 6 of the 16 non-shooters were construction workers, and 2 of the remaining 10 were spectators (non-shooters) in the vicinity of the discharge area.

The elements detected on the hands of non-shooters above the signal threshold from the blank cotton swabs included Cu, Fe, K, S, and Zn.

An intraday variation study was performed with 2 of the non-shooters whose hands were swabbed three times at different hours of the day (morning, afternoon, and

evening). The results demonstrated that concentrations of Cu, K, and Zn can vary for one person, which can be related to everyday activities. The presence of Fe was detected in one of the non-shooters on their right hand. Iron was not observed for the other 10 non-shooters.

The elements detected on the hands of the 6 construction workers included: Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, S, Si, Sr, Ti, and Zn. Figure 24 shows the distribution of some of these elements on the left and right hands.

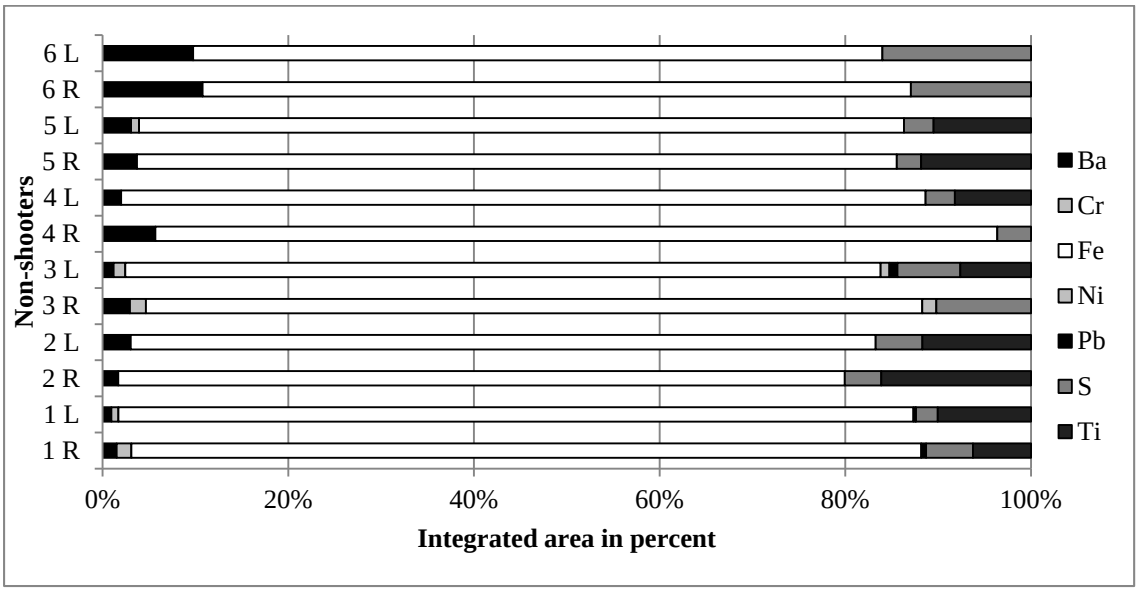

Figure 24. Distribution of some elements detected on the left (L) and right (R) hands of non-shooters working in construction

Target elements (Ba, Pb) in GSR analysis were detected on the hands of two of the non-shooters. The concentration of Ba was 0.1ppm on the left hands of two non-shooters; this value was above the concentration found in cotton swabs. Also, Pb was

detected at concentrations of 0.3 ppm, 0.8 ppm, and 1.2 ppm on the hands of non-shooters number 1 (R and L) and number 3 (L), respectively.

5.2.6 Results for the detection of elements on the hands of shooters

5.2.6.1 Results from LIBS analysis

One of the objectives of the present study was to determine the capability of LIBS to detect elements characteristic of GSR presence. For the purpose of evaluating the capabilities of LIBS to detect GSR, the upper area of the right and left hands of a total of 43 officers were swabbed before and after shooting each type of ammunition. A total of 153 hand swab samples were collected from police officers for LIBS analysis.

The elements detected on the hands of the officers above the cotton blank signal threshold included: Ba, Cu, Fe, K, Ni, Pb, and Sr. Figure 25 shows the amount of elements extracted from the bulk hand swab samples from the left and right hands of shooters. For simplicity, all the elements except K are shown in the figure. The amount of K extracted from the cotton swabs from pistol, rifle, and shotgun shooters was 13347 ±9790 ng, 11342 ±7017 ng, 16727 ±7057 ng, respectively.

Overall, quantitative analysis of the elements detected on the hands of shooters does not show clear differences between pistol, rifle, and shotgun shooters. However, qualitative examination shows the detection of Fe only for shotgun shooters, and detection of Pb only on pistol and rifle shooters.

Elements characteristic (Ba, Pb) of GSR presence were detected in the hands of shooters. According to the results obtained, Ba was detected on either the left or right hands of all officers. The error bars for Ba in Figure 25 is a representation of the wide

concentration ranges detected on the hands of the shooters; although in the negative range of the graph the error bars do not represent negative values in the data. On the other hand, Pb was only detected on the hands of pistol and rifle shooters with signals close to the detection limit.

Another important element in the identification of GSR is Sb. In this study Sb was not detected in any of the samples from shooters. The possible reason for this is a combination of Sb concentrations below the method detection limit and peak overlap with Fe emission line at (II) 259.9 nm.

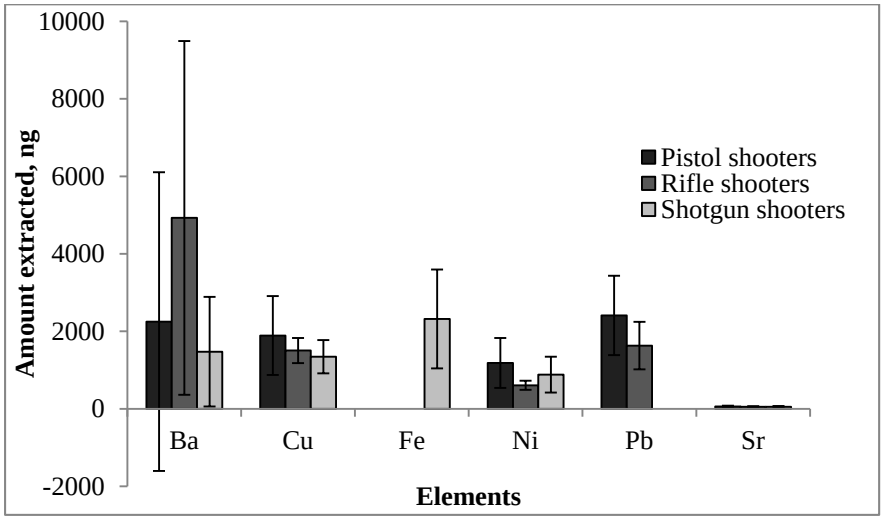

Figure 25. Average amount of elements extracted from cotton swab samples of pistol, rifle, and shotgun shooters (left and right hands)

5.2.6.2 Results from ICP-OES analysis

Cross validation of LIBS results were performed by solution ICP-OES because this is a more mature and sensitive technique which is suitable for analysis of trace

elements. A total of 138 hand swab samples from police officers were analyzed by solution ICP-OES.

The elements detected by ICP-OES on the hands of shooters were: Al, Ba, Ca, Cu, Fe, K, Mg, Na, Ni, Pb, S, Sb, Sr, and Zn. The detection of these elements confirmed the results obtained by LIBS.

Quantitative analysis of all elements was performed using the regression results for each element. The discrimination power of the elements detected to differentiate shooters from non-shooters will be examined in Chapter 6 using statistical tools.

Analysis by solution ICP-OES allowed confirmation for the identification of GSR on the hands of shooters. In order to examine differences between concentration of Ba, Pb, and Sb on right versus left hand samples, a small sample group of 10 officers were selected from the data. Figure 26 is a visual representation of the amount extracted from cotton swabs for the left and right hands of 10 officers.

From the graph, there is not a clear trend on whether sampling from the right hand (sometimes referred to as the shooting hand) or the left hand will provide more information of the elemental composition in the samples. Although the data presented here is not intended to be a representation of the population as a whole, it is observed that in 5 out of 9 times (56%), detection of Sb was observed in the right hand of shooters. Therefore, there are similar possibilities of finding Ba, Pb, and Sb on either hands of a shooter.

The graph in Figure 26 is further divided into pistol, rifle, and shotgun shooters. From this small group of 10 officers it is evident that Sb was detected in all of the

shooters with rifle. Concentrations from Ba and Pb varied and did not show a clear trend for these samples.

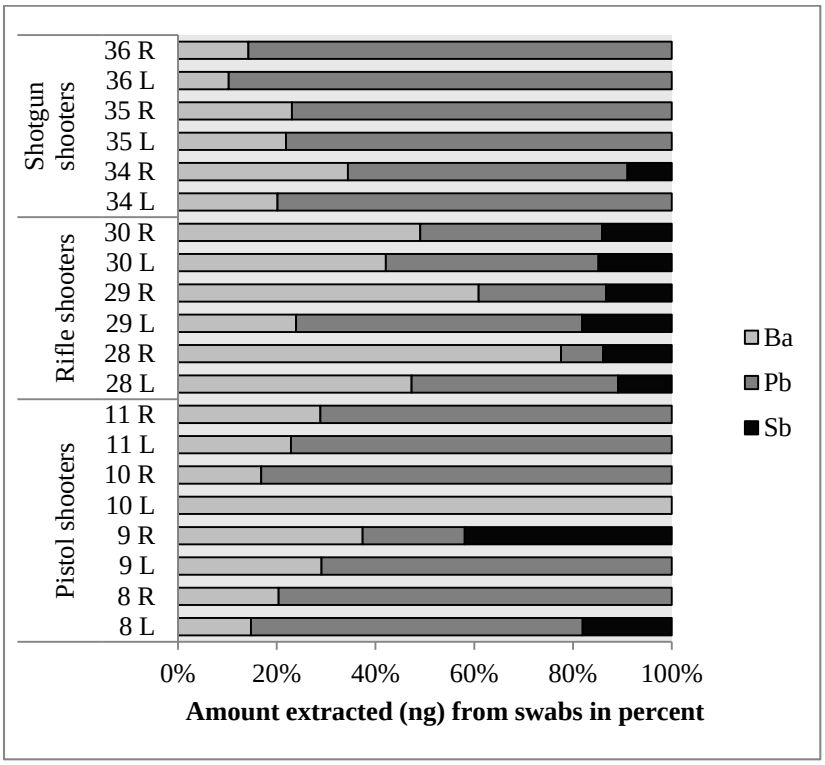

Figure 26. Detection of Ba, Pb, and Sb on the left (L) and right (R) hands from 10 officers. The number system in this graph can be traced to the organic analysis results in the previous chapter (Section 4.2.4)

A comparison of the results obtained from the right hands of all shooters is summarized in Figure 27. The graph is also divided into groups of pistol, rifle, and shotgun shooters. Similar to the previous example, the concentration range of Ba, Pb, and Sb varies within all the samples without a clear trend. In addition, Sb was detected more times in pistol and rifle shooters than in shotgun shooters.

The results obtained here cannot be generalized and the same trend may not be observed for other sample sets.

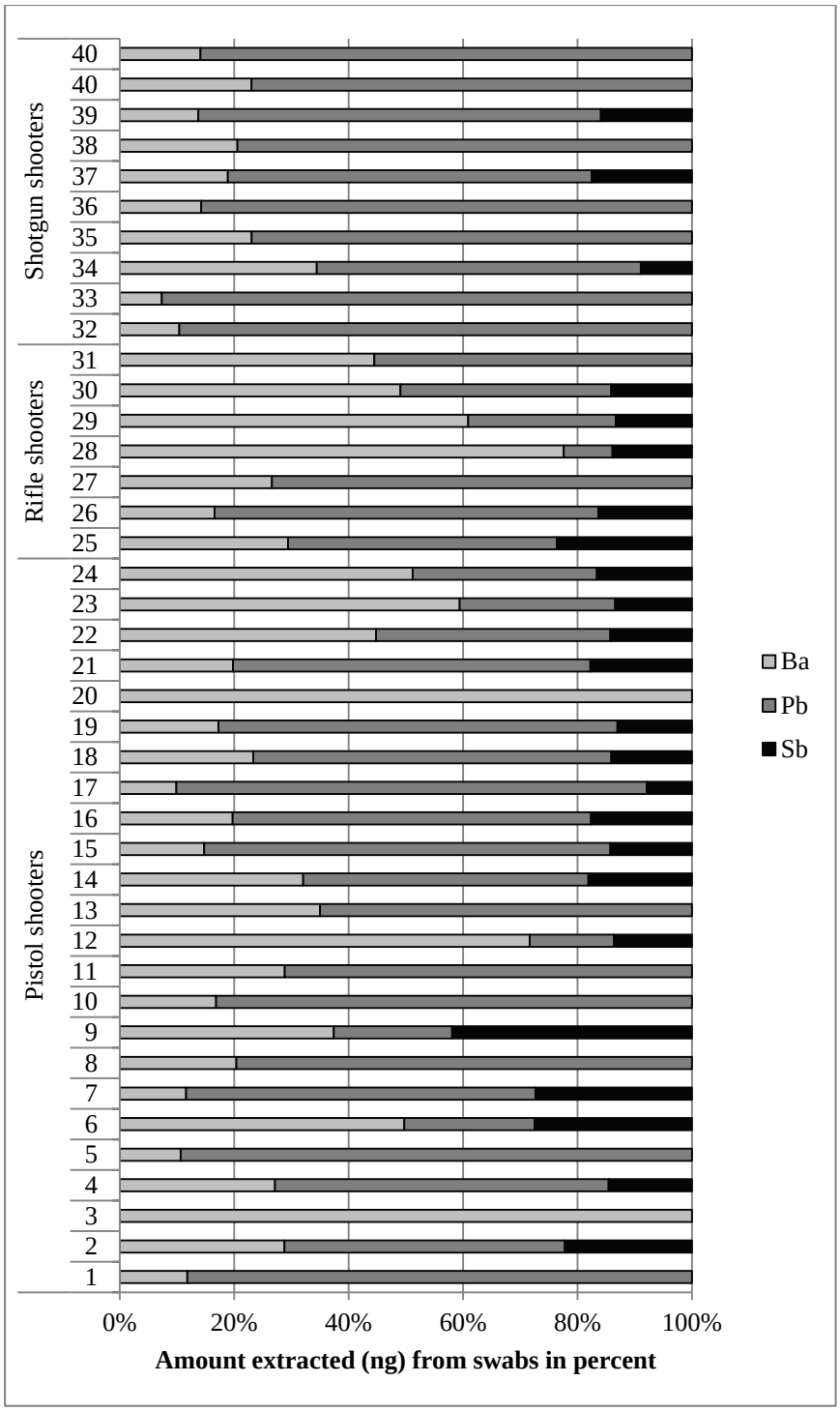

Figure 27. Summary of results for the detection of Ba, Pb, and Sb on the right hands of shooters

5.2.7 Results for the detection of elements in spent cartridges

Analysis of the inorganic components that remain in the spent cartridges after the discharge was performed to determine which elements can potentially be transferred to the hands of shooters. The inside walls of the cartridges were swabbed followed by the extraction procedure described for hand swab samples.

Table 11. Elemental composition present in the primer mixture of different types of ammunition used by shooters in the study

Elemental composition	Ammunition		
	Pistol and rifle		Shotgun
	Winchester® % by Weight	American Eagle® % by Weight	Federal Premium® % by Weight
Cu	55-96	54-86	0-75
Zn	10-55	3-37	0-5
$Ba(NO_3)_2$	3-3.5		
Ba		1-8	
Sb_2S_3	1-5	0.5-4	
Sb			0-5
Pb			0-75
$Pb(SCN)_2$	0.1-0.6		
Lead styphnate	4-5		
Lead, dihydroxy[2,4,6-trinitro-1,3-benzenediolato(2-)]di-		2-8	
Ni		0-1	0-6
Al		0.1-2	
Fe			0-75
W			0-60
Sn			0-6

* Concentration range for all types and brands of ammunition (Section 5.1.4.3)

Table 11 shows the element compositions present in the primer mixture of the different types of ammunition. Except for tungsten (W), all elements listed in Table 11 were monitored by both LIBS and ICP-OES.

The detection of Ba, Pb and Sb by LIBS was possible for the three types of ammunition, pistol, rifle, and shotgun. The analysis of the inorganic components in GSR from spent cartridges, allows the characterization of elements present in GSR. Figure 28 summarizes the amount of each element detected by LIBS above the MQL.

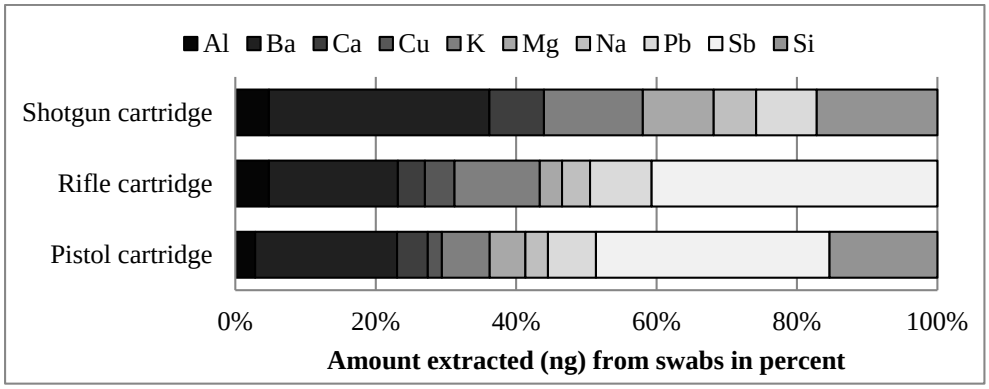

Figure 28. Summary for the detection of elements present in spent cartridges from pistol, rifle, and shotgun ammunition results by LIBS

5.2.8 Evaluation of the significance of the elemental analysis of GSR

The goal of this study is to combine information from inorganic and organic analysis in order to provide unambiguous identification of GSR. The present study demonstrates evidence on the importance of performing elemental analysis of GSR samples.

In Chapter 4, the organic analysis from swab samples from 40 shooters was represented in a graph (Figure 15, Section 4.2.4). For 7 shooters (numbers 10, 13, 20, 24, 28, 29, and 31) neither NG nor DPA were detected in the samples. Examining the elemental analysis results from ICP-OES for these same samples, only Ba was detected in number 20, Ba and Pb were detected in 10, 13, and 31, and a combination of BaPbSb was

detected in 24, 28, and 29. For number 20 examination of the left hand of the shooter shows the presence of Ba and Pb in the sample. Similarly results for number 3 show the detection of Ba only, however, NG was also detected on the sample.

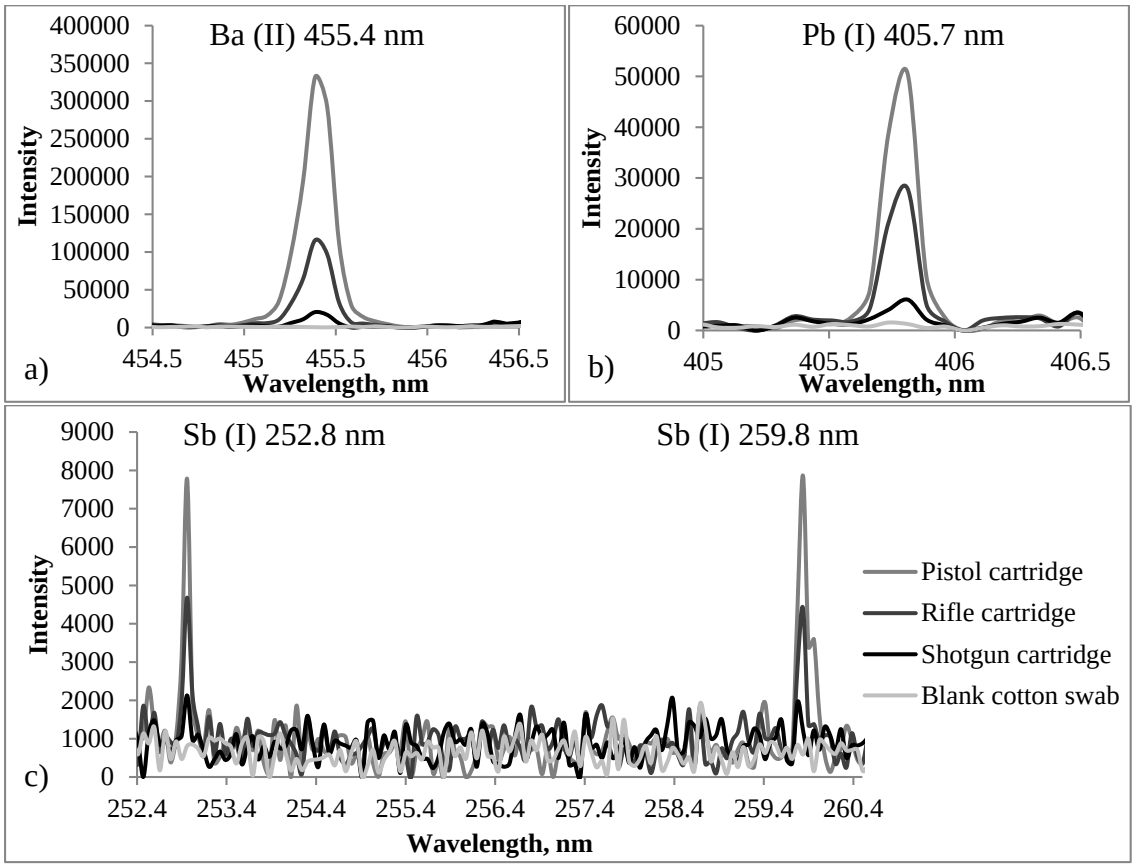

Figure 29. LIBS spectra for a) Ba (II) 455.4 nm, b) Pb (I) 405.7 nm, and c) Sb (I) 252.8 nm and (I) 259.8 nm detected in spent cartridges

Further evaluation of organic and inorganic analysis will be performed through statistical analysis to demonstrate the advantages of performing both organic and inorganic analysis of GSR.

Unlike samples collected from the hands of shooters, the emission lines for Sb were detected by LIBS, for pistol and rifle ammunitions. The presence of Sb was confirmed with the three emission lines for Sb (259.8 nm, 252.8 nm, and 323.2 nm).

5.3 Conclusions for the elemental analysis of hand swabs by LIBS and ICP-OES

The capabilities of LIBS were evaluated for the analysis of GSR samples from the hands of shooters. Qualitative and quantitative methods were developed and optimized for the analysis of elements expected to be present in GSR samples.

Several methods were evaluated for the analysis of GSR on cotton swabs by LIBS. The use of Teflon as a supporting material to spike the samples resulted to be the most appropriate method producing low background and minimal interferences during ablation. The use of solution ICP-OES as a confirmatory technique allowed the characterization of GSR on the hands of shooters.

The elemental profile of blank cotton swabs was investigated to identify the elemental contribution of the cotton matrix to GSR samples. Results indicated that swabbing with cotton applicators is an effective method for GSR collection and there were significant differences between the elemental profile of cotton and that of hand swab samples. Using ICP-OES, the presence of Ba was detected on blank cotton swabs. However, the amount of Ba present in the blank cotton swabs was lower than the amount detected in samples from shooters and non-shooters.

A control group study was conducted with 16 non-shooters to characterize the samples and find differences between shooters and non-shooters. The presence of Ba and Pb was detected in two of the non-shooters working in construction and Ba was present

in one non-shooter. These results confirm published studies in which elements consistent with GSR presence have been found on the hands of individuals (non-shooters) [Garofano et al., 1999; Grima et al., 2012]. Furthermore, the probability of finding Ba and Pb on the hands of non-shooters demonstrate the need to perform organic analysis of GSR samples.

As a proof of concept, swab samples from the right and left hands of officers were collected after shooting in an open range, for analysis by LIBS and ICP-OES. In all the samples Ba was detected and Pb was detected in some occasions. The method detection limits for Sb by LIBS compared to concentrations found in GSR by ICP-OES analysis suggest that the detection Sb is limited. In addition, interferences with Fe emission line (259.9 nm) was observed when analyzing swab samples from shooters. Other emission lines for Sb were not found in the LIBS spectra even at high concentrations (300 ppm). However, confirmation by solution ICP-OES for Sb on the hands of shooters was possible. For shotgun shooters, Sb was not detected in most of the samples, thus the MSDS for ammunition components was used to propose a possible cause. As indicated by the MSDS for shotgun ammunition, some mixtures may not contain Sb.

To conclude, this study provides indication of the capability of LIBS for the detection of GSR on the hands of shooters. The fast results obtained by LIBS indicate the suitability of this technique as a screening tool, and the availability of portable LIBS systems offers the potential for GSR identification in the field.

6 DATA FUSION OF INORGANIC AND ORGANIC COMPONENTS IN GSR

6.1 Multivariate analysis

Multivariate analysis is the simultaneous examination of more than one statistical variable [Miller 2005]. This type of statistical analysis is particularly important to determine the interactions of multiple variables in a measurement. In analytical chemistry, for instance, the interactions of different species (i.e. elements) measured simultaneously in a single sample by a sensor (i.e. spectrometer) is of particular importance. If more than one variable is measured for a single sample the combination of these variables can be define as multivariate data.

There are different methods and techniques available to perform multivariate analysis. In the following sections, techniques used in multivariate analysis within the scope of this dissertation will be described.

6.1.1 Principal component analysis (PCA)

One of the disadvantages with multivariate data is that the large amount of variables can prevent the recognition of a pattern or relationship in the data.

Principal component analysis (PCA) is a multivariate analysis technique used to determine the relationship between correlated variables [Miller 2005]. The primary aim of PCA is to reduce the number of variables while accounting for the majority of the original variation in the data [Chatfield 2000].

Variable reduction in PCA is performed by transforming a set of correlated data into a new set of uncorrelated data with decreasing variance. This transformation is performed by calculating principal components (PC). Principal components are a linear

combination of the original variables. The first principal component represents the most variation, which decreases successively with each PC [Miller 2005].

In mathematics, each principal component is an eigenvector with a corresponding eigenvalue, which represents the amount of variance in the data explained by the principal component. The eigenvector with the largest corresponding eigenvalue is the first principal component. Each variable contributes to the magnitude of different eigenvectors in a non-proportional manner. As a result, this information can be used to determine which variables account for the variation in the data [Miller 2005].

The graphical representation of the PCA is called a score plot. Each principal component is orthogonal (i.e. right angle) to each other in the score plot. A particular sample is represented with as many scores as principal components are retained. A score is a value that represents the influence the principal components has on the sample. The score plot can be bidimensional (2D), when two principal components are plotted or tridimensional (3D), when three principal components are plotted. A significant distance within two groups is observed if the samples are separated in the y or x-coordinate from the origin or in a diagonal line (y=x).

6.1.2 Partial least squares discriminant analysis (PLSDA)

Partial least squares discriminant analysis (PLSDA) is a regression model that shares similar principles as PCA. In both methods, principal components are calculated to reduce the number of variables to represent the data. Using the first few PCs should provide the greatest information about the data [Martens 2001].

The main difference between PLSDA and PCA is that the former uses predictor variables to calculate the PC. In PLSDA variables are divided into predictor variables and response variables. These two groups of variables represent different properties of the sample (i.e. concentration vs. instrument response). To calculate the PC in PLSDA the predictor variables are used and as a result the number of variables can be reduced. The PCs are selected in such a way that the predictor variables describe most of the variation in the data as possible [Miller 2005].

6.1.3 Linear discriminant analysis (LDA)

Unlike PCA, linear discriminant analysis (LDA) is a supervised pattern recognition method because the relationship of the sample to a group must be known. For instance, in this work the samples can be divided into two known populations, shooters and non-shooters. The aim of LDA is to create a model using rules to allocate a new sample to the correct group (i.e. shooter or non-shooter).

The first step in LDA is to find a linear discriminant function (LDF), which is a linear combination that represents all the original variables with a single value, Y. The LDF is calculated in such a way that each group will have very different Y values [Miller 2005].

The second step in LDA is to determine the success of the model using different tests. One method randomly divides the data into two groups. The first group is the training set and is used to calculate the LDF. The second group is the test set and each sample in the test set is allocated to a particular group within the training set. A success rate for the model is found to indicate the correct association (%CA) of the test set.

A second method to test the LDA model is cross-validation or the leave one out method. As the name implies in this validation test, the LDF is found with all the samples except for one sample which is omitted. Then the omitted sample is allocated to a group. This process is repeated for all the samples in the data and a success rate is then found.

6.1.4 K-nearest neighbor (KNN)

The K-nearest neighbor (KNN) is a simple method for allocating a sample to the correct group. The KNN method can be used when there are two or more groups in the data that cannot be separated in a 2D plane [Miller 2005]. To use this method there is a training set that represent all the groups in the data. A test set is used to test the model into correctly associating each sample to a particular group.

6.2 Experimental

6.2.1 Instrumentation

The analysis of target VOCs extracted with CMV devices from cotton swab samples was performed with the GC-MS (Agilent Technologies, Santa Clara, CA) GC system 7890A and a GC/MS Single Quad 5975C, as described in Section 4.1.1.1. The GC system is equipped with a Thermal Separation Probe (TSP) (Agilent Technologies, Santa Clara, CA) to thermally desorb the CMV devices into the GC-MS injector.

The analyses of target elements were conducted on a LIBS J200 system (Applied Spectra, Freemont, CA), equipped with a 266 nm ns-Nd:YAG laser, as described in Section 5.1.1.1. The ICP-OES analysis was conducted on the Optima 7300DV

(PerkinElmer, Waltham, MA) integrated with an Echelle spectrometer and a segment charge coupled device (SCD) detector, as described in Section 5.1.1.2.

6.2.2 Samples from shooters, non-shooters, and spent cartridges

Multivariate analysis was performed for the data collected by LIBS, ICP-OES, and GC-MS using optimized instrument and analyses parameters. The population number used in the statistical analysis depended on the requirements to perform the multivariate analysis.

For multivariate analysis of LIBS data a total of 366 samples including replicates from shooters and non-shooters were used for PCA and LDA. Multivariate analysis by PLSDA and KNN was performed with 326 reference samples and 30 test samples treated as unknowns. The samples used in PLSDA were the same samples analyzed by GC-MS, as required by the statistical model.

For statistical analysis of ICP-OES results a total of 750 samples were used from shooters and non-shooters for PCA and LDA.

Multivariate analysis for GC-MS results by PLSDA and KNN was performed with 66 reference samples and 30 test samples treated as unknowns. The samples used in PLSDA were the same samples analyzed by LIBS, as required by the statistical model.

Data fusion of LIBS and GC-MS data was performed using PLSDA results with the FIACS software created for data fusion of ink samples analyzed by different instruments [Trejos et al., 2015]. For this model a total of 284 samples from LIBS and GC-MS analyses were used as the reference and a total of 60 samples were used as the test samples.

A total of 45 data samples from spent cartridges were used in the PCA for ICP-OES results and a total of 9 data samples were used in the PCA for LIBS results.

6.2.3 Data reduction and statistical analysis

Data reduction and statistical analyses were performed with MSD ChemStation data analysis software (v E.02.01.1177 Agilent Technologies, Santa Clara, CA), Aurora LIBS data analysis software (v 2.1, Applied Spectra, CA), WinLab32 (PerkinElmer, Waltham, MA, USA), Microsoft Excel 2010 (v 14.0.7153.5000, Microsoft Corp., Redmond, WA), JMP (v 12.1.0 SAS, NC), and the Forensic Ink Analysis and Comparison System (FIACS) (CoVar and Applied Spectra).

6.3 Results and discussion

The goal of this work is to provide unambiguous identification of GSR on the hands of a shooter. A simpler data fusion model (i.e. data tables) was used in previous chapters to compare the presence of inorganic components (Ba, Pb, and Sb) and organic components (NG and DPA) characteristic of GSR presence on the hands of shooters.

In this chapter more complex statistical models are used to analyze the multivariate data obtained by LIBS, ICP-OES, and GC-MS. The purpose of statistical analysis in this study is to find correlations between samples from the same group (i.e. shooters) using all the measured variables. Successful association of samples into the corresponding groups will demonstrate the utility of multi-elemental analysis for GSR.

6.3.1 PCA and LDA for LIBS results

For multivariate analysis of LIBS data a total of 366 samples including replicates from shooters (n=326) and non-shooters (n=40) were used. The data used for PCA and LDA consisted of the integrated area for each element. A value of zero (0) was assigned for variables in the samples where the integrated area for a particular element was below the method detection limit or SNR < 3.

The elements used for statistical analysis were: Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Na, Ni, Pb, Sr, and Zn. Initially all the variables were used in the PCA, which allowed the examination of the data and determination of correlation between the variables. From the first analysis several elements were removed according to the low contribution for explaining the data. The elements removed were: Ca, Cr, K, Na, and Zn.

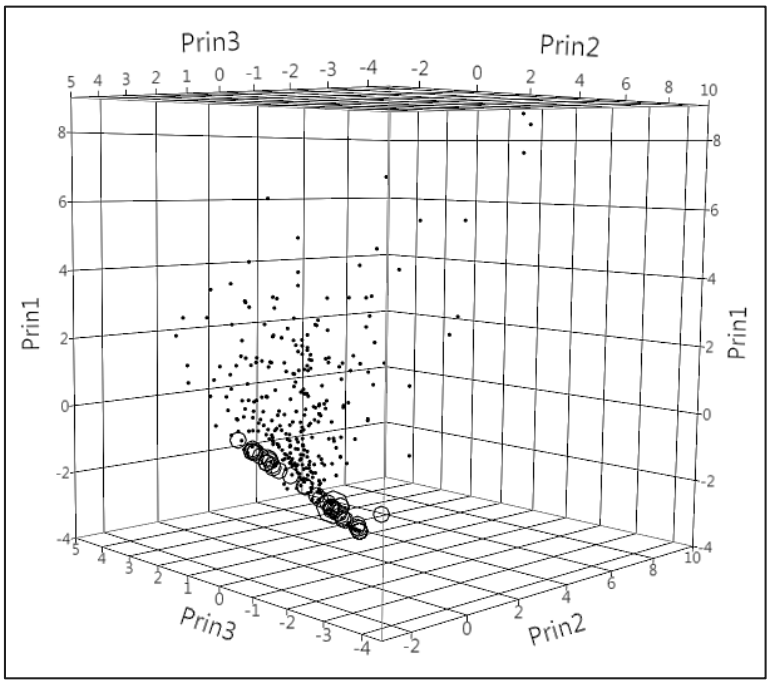

Figure 30. PCA score plot for LIBS data, represented in blue are the non-shooters and the red group are the shooters

The new PCA results yield values for the first 3 PCs that accounted for 83.3% of the variation in the data. The most important variable in PC1 according to the score was Pb. Figure 30 shows the score plot for the 3 PCs (Prin1, Prin2, and Prin3) where the open circles represent the non-shooters and the dots represent the shooters. Examination of the score plot shows poor separation between the two groups. However, the scores for non-shooters are clearly grouped together along the PC3 axis.

Analysis of the data using LDA was conducted to determine if LIBS data could provide good association between the groups. The data was divided into two groups: shooters and non-shooters. A correct association of 88% was obtained for non-shooters and 76% for shooters. From the non-shooters, 5 samples were misclassified, examination of the samples showed that this samples belonged to non-shooters who were spectators close to the area of discharge.

From the shooters, 77 samples were misclassified, 10 of samples belong to the shooters instructors, who were not shooting during sample collection. From the remainder 67 samples, 40 samples were misclassified which belong to samples from the left hand of shooters. The last 27 samples that were misclassified belonged to samples from the right hand of shooters. The misclassified samples were one or two replicates of a sample that were not associated with the group because of absence of Ba or lower intensity for elements present on the hands of shooters.

6.3.2 PCA and LDA for ICP-OES results

For statistical analysis of ICP-OES results a total of 750 samples were used from shooters (n=520) and non-shooters (n=230).

The elements used for statistical analysis were: Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Sb, Si, Sr, Ti, and Zn. Initially all the variables were used in the PCA, which allowed the examination of the data and determination of correlation between the variables. Some separation of the groups was observed, however several elements were removed according to the low contribution for explaining the data. The elements removed were: Ca, Cr, Fe, K, Na, P, and Zn.

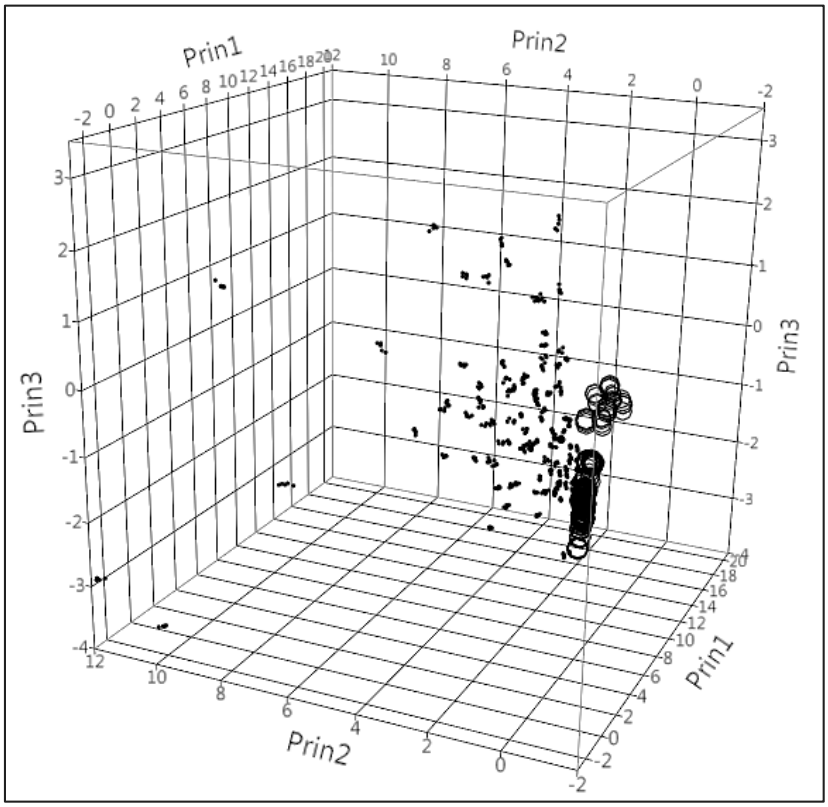

Figure 31. PCA score plot for ICP-OES data, represented in blue are the non-shooters and the red group are the shooters

The new PCA results yield values for the first 3 PCs that accounted for 82.6% of the variation in the data. Figure 31 shows the score plot for the 3 PCs (Prin1, Prin2, and

Prin3) where the open circles represent the non-shooters and the dots represent the shooters. The most important variables in PC2 according to the scores were Ba, Pb, and Sb. Hence, the separation of the shooters group is observed along PC2.

The score plot was rotated in such a way that maximizes visual representation of the separation between the two groups. Even though the percent of variation for ICP-OES was lower than that obtained for LIBS data, the variables analyzed by ICP-OES allowed the separation of the two groups. Similar to the score plot for LIBS data, Figure 31 shows grouping of non-shooters along PC3 and wide scattering of the scores for shooters.

Analysis of the data using LDA was conducted to determine if ICP-OES data perform better than the previous analysis with LIBS data. The data was divided into two groups: shooters and non-shooters. A correct association of 91% was obtained for non-shooters and 97% for shooters. From the non-shooters, 20 samples were misclassified, these were the same samples misclassified by LIBS, which belonged to non-shooters who were spectators close to the area of discharge.

From the shooters, 15 samples were misclassified; these samples included replicates and corresponded to 3 different shooters. Manual examination of the elemental menu showed that Ba, Pb, and Sb were present in these samples. Therefore, misclassification resulted from the absence or low concentrations from other elements in the samples.

6.3.3 KNN results for LIBS and GC-MS

Multivariate analysis by KNN was performed with 326 reference samples and 30 test samples treated as unknowns from LIBS analyses, and 66 reference samples and 30

test samples from GC-MS analyses. From the 30 test samples from LIBS and GC-MS 26 were from shooters and 4 from non-shooters.

For KNN analysis, the data was in the form of spectra for LIBS and the chromatograms for GC-MS. The software used k=10, thus the spectra or chromatograms were compared to the closest 10 spectra or chromatograms from the reference. A correct association (CA) of 100% resulted for the shooters and the non-shooters from GC-MS data. For LIBS data 100% CA was obtained for shooters, and 75% for non-shooters. One replicate from a non-shooter was associated with a shooter. Examination of the LIBS spectra indicates that Ba and Pb were not detected on the hands of this person, thus this person should have been classified as a non-shooter.

6.3.4 Data fusion from LIBS and GC-MS results using PLSDA

Multivariate analysis by PLSDA was performed with the same samples used for KNN. An example of the output of PLSDA by the FIACS software is shown in Figure 32. The software consists of three plots one for each technique studied and the last one for the fusion of the data. A menu on the right hand side has a list of the test samples (n=30). At the top of each plot is a list of 5 reference samples that match the test sample. The larger the bar on the plot, either on the positive or negative range, the more association is found with that reference sample.

The correct association rates are calculated with respect to the first sample on the list (the larger bar). A correct association (CA) of 100% resulted for the shooters and 50% for the non-shooters. The two samples misclassified were from the non-shooter who

was in the area of the discharge. These are the same samples that were misclassified previously by LDA.

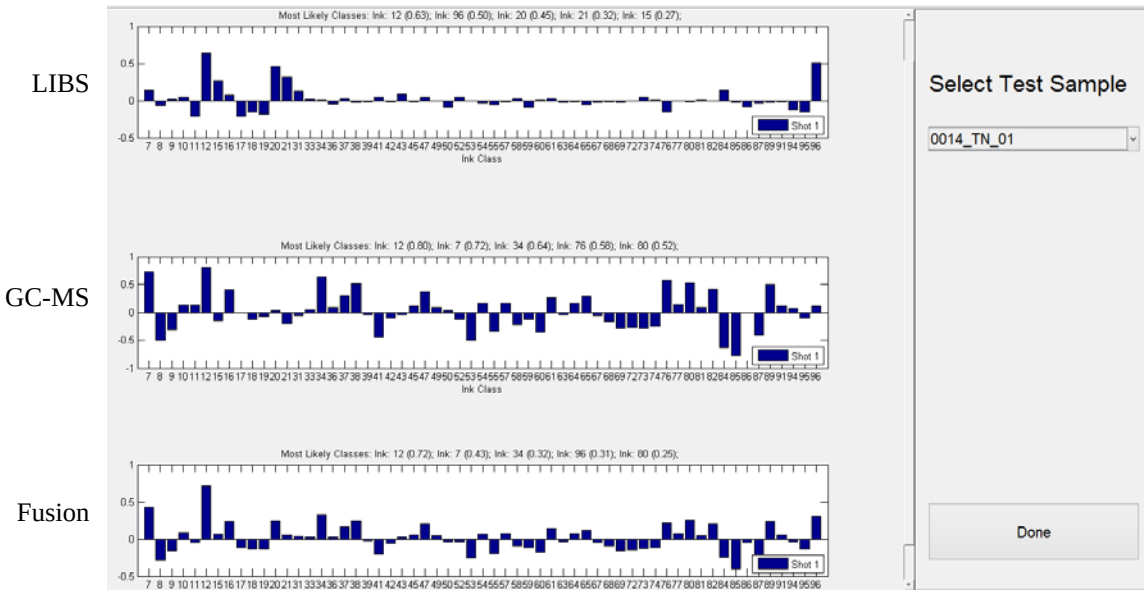

Figure 32. Example of PLSDA output with the FIACS software, evaluating results from a shooter

6.4 Conclusions for multivariate analysis and data fusion of inorganic and organic analysis

The statistical analysis of inorganic and organic composition of samples from shooters and non-shooters was performed to determine the significance of the analyses. In addition, statistical analysis was used as a tool to determine whether grouping and association to a group could be achieved. The ultimate goal of the statistical analysis was to demonstrate improvement in association rates by fusing organic and inorganic data.

Four different statistical tools were evaluated in this study: PCA, LDA, KNN, and PLSDA. The PCA resulted in separation of shooters and non-shooters with ICP-OES

data. Visual separation of groups was expected because ICP-OES is more sensitive to provide better differentiation in elemental profile and it allows the detection of all the characteristic elements (i.e., Ba, Pb, and Sb) in the shooters samples.

Similarly, LDA was performed to determine the capability to associate samples to the correct groups (shooter or non-shooter). The LDA association rates were higher for ICP-OES data than for LIBS data. Nonetheless, LIBS data provided relatively good correct association rate for non-shooters samples were only the samples from one non-shooter was misclassified, but elemental analysis showed that Ba, Pb, or Sb were not present in that particular sample.

Another method used for associating a sample to the corresponding group was KNN. In contrast to LDA were a linear function is created for the different groups, KNN compares the unknown sample to the closest matching reference samples. The results by KNN provided good association rates when comparing the spectra from LIBS and GC-MS separately. The 100% association rate achieved for GC-MS results, demonstrated that there is a distinctive chromatographic profile for shooters and non-shooters.

The ultimate goal of this study is to provide unambiguous identification of GSR on the hands of a shooter. To achieve this goal fusion of organic and inorganic data was performed with computer based software that employs PLSDA for statistical analysis. A correct association rate of 100% for shooters was achieved by PLSDA, while a 50% correct association was achieved for non-shooters. It was demonstrated that the results obtained by data fusion do not improve the identification of shooters and non-shooters compared to statistical analysis with KNN.

7 OVERALL CONCLUSIONS

The capabilities of different techniques for field analysis of inorganic and organic matrixes were evaluated. Important characteristics that a technique should have for field analysis include: portability, robustness, and relatively good sensitivity for the detection of the target species. Both of the techniques selected for the present work, LIBS and CMV-GC-MS, meet the requirements to be used as portable devices. In fact, there are commercially available portable systems for LIBS and GC-MS analysis.

A fast and portable device was evaluated for the first time for the extraction of organic compounds from the headspace of GSR samples and compounds present in contaminated air. The Capillary Microextraction of Volatiles (CMV) device has demonstrated to be a fast and sensitive technique for the headspace extraction of volatile organic compounds with a wide range of physical properties.

The utility of CMV devices for the analysis of VOCs in ambient air was demonstrated. An indirect comparison of the performance of CMV with sorbent tubes for extraction and detection of a set of important VOCs was conducted by following the criteria specified in the EPA method TO-17 for the analysis of ambient air using the CMV and comparing to previously reported results for the sorbent tubes. The overall results for headspace extraction with CMV demonstrated that a) faster extraction of air samples (<10 min) can be performed compared to sorbent tubes (>1 hour of sampling), b) low detection limits can be achieved (~5 ng) for most compounds, and c) good replicate precision can be achieved.

The results obtained from indoor air samples demonstrated the suitability of the CMV for air monitoring. The chromatograms obtained from the hair and nail salon show

high signal intensity for compounds commonly found in cosmetic products (i.e., nail lacquer and polish, and polish remover). Although many of the compounds can be sensed through smell, the importance of the study was to identify odorless compounds that can have acute or chronic effects to human health. While there are regulations for air contaminants in the workplace, there is not an air quality index for indoor air. Therefore, the CMV can be potentially used for monitoring air quality in rooms.

The major advantages of CMV demonstrated through this study included: a) the ability to use large sampling flow rates with short extraction times (<2 mins), b) cost efficiency, which allows the devices to be disposable, and c) capability of multiple uses without losing extraction efficiency.

In the second part of the study, the capabilities of CMV-GC-MS and LIBS were evaluated for the detection of the organic and inorganic composition of GSR, respectively. The aim of this study was to demonstrate the utility of these techniques for field analysis with currently commercially available portable systems.

The analysis of gunshot residue has been traditionally performed by SEM-EDS, which is a mature technique that allows both morphological and elemental analysis of GSR particles. The main disadvantage of SEM-EDS is that it is time consuming, taking up to 8 hrs to analyze one sample, and identification relies in the detection of small particles < 10 μm that can be mask by skin debris from the suspect. Therefore, there is a need to advance the analysis of GSR using techniques that can provide fast and unambiguous identification. The capabilities of LIBS for elemental analysis of GSR were demonstrated and discussed in this work. It was shown that although signal interferences may occur for Sb emission lines, LIBS can be used as a fast screening tool for GSR

detection. The availability of LIBS as a commercially portable system shows the importance of this study for future developments in field analysis of GSR.

The capabilities of CMV were evaluated for the first time, for the headspace extraction of target compounds from GSR samples. The overall results showed that DPA and NG, two organic compounds present in smokeless powders can be extracted and detected from GSR samples using the CMV coupled to a GC-MS and μECD detector. The use of CMV for the detection of organic compounds is an attractive alternative because it is a nondestructive method, which permits further analysis of the sample. The results presented here demonstrate the importance of analyzing the organic components of GSR as a means to obtain more information from the sample.

In a typical forensic case, the analysis of both the inorganic and organic components could be combined for unambiguous identification of GSR on the hands of shooters. The use of CMV for analysis of organic components in GSR is an attractive, nondestructive method that can be used in combination to currently used methods (i.e., SEM-EDS analysis).

One of the objectives of the study was to provide unambiguous identification of GSR on the hands of shooters by combining the analysis of both organic and inorganic components. Manual examination of results, such as identification of target compounds and characteristic elements (Ba, Pb, Sb, NG, and DPA), demonstrated the advantage of combining the information obtained from the sample. Nonetheless, the chemical profile obtained by LIBS and GC-MS was evaluated through statistical analysis and data fusion techniques.

Four different statistical tools were evaluated in this study: principal component analysis (PCA), linear discriminant analysis (LDA), K-nearest neighbor (KNN), and partial least squares discriminant analysis (PLSDA). The PCA was used as a visual representation of the elemental profile obtained by LIBS and ICP-OES. Visual separation of groups from shooters and non-shooters was achieved with ICP-OES data. The groupings obtained were mainly attributed to the presence of Ba, Pb, and Sb on the hands of shooters, represented by PC2. The LDA method demonstrated good performance for correctly associating the training samples to the respective groups with LIBS and improvement was observed with ICP-OES data.

Overall, the KNN and PLSDA statistical analysis tools provided good association rates when comparing the spectra from LIBS and chromatograms for GC-MS. The PLSDA was used as a data fusion tool to combine the information obtained from LIBS and GC-MS. However, 100% correct association was obtained with both KNN and PLSDA, when analyzing the GC-MS chromatograms. Therefore, data fusion did not provide improvement in the identification of shooters.

7.1 Future research work

The results obtained in this study demonstrated the potential to improve headspace extraction by CMV and data analysis.

The PDMS coating in the PSPME that makes up the CMV is a universal absorbent material. However, the low retention of some compounds by the PDMS demonstrates the need for improving the coating of the PSPME. Future development of PSPME will include the addition of carbon particles (e.g., Carboxen®) to the PDMS

coating to improve retention of smaller compounds, such as methylene chloride or more polar compounds such as phenol. In addition to the retention capability, the modification of the PSPME coating is expected to significantly reduce the high breakthrough currently observed for the compounds selected in the study. Also, by reducing the breakthrough, faster extraction times (~ 2 mins sampling time) can be envisioned for this technique.

Furthermore, the analysis of volatile organic compounds in samples from the hands of shooters showed the presence of multiple chromatographic peaks that can provide further information for the identification of a shooter. For the purpose of the current study, only two compounds were monitored, NG and DPA. However, the chromatogram of the samples shows information that aided in the correct association (100%) of shooters when using statistical analysis tools. Therefore, the identification of other compounds on the hands of shooters could provide additional information.

Finally, the observations and results gathered in this work provide important information for the development of field analysis studies. The CMV is a portable sampling technique that could be potentially coupled to a portable GC-MS system. Similarly, portable LIBS systems are commercially available. Future method developments with both techniques could provide detection of GSR and identification of shooters in the field.

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