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Detection of organic gunshot residue using High Performance Liquid Chromatography/Mass Spectrometry

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DETECTION OF ORGANIC GUNSHOT RESIDUE USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY

by

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ABSTRACT

Gunshot residue (GSR) has been analyzed in forensic laboratories since 1933 when the dermal nitrate test originated. (1) Detection and analysis of GSR has since developed with the invention and implementation of instrumentation. Since the 1960s, inorganic gunshot residue (IGSR) has been the primary focus for GSR analysis. (2) As disadvantages like omitting lead from ammunition and the transient properties of IGSR are researched, it is clear that a new approach is needed. Organic gunshot residue (OGSR) analysis has the potential to become the novel approach for GSR analysis because OGSR does not have the same transient properties as IGSR. (3) The compounds are lipophilic and are therefore more likely to remain on the shooter's hands or face. (4) OGSR can be analyzed through a myriad of instrumentations, including High Performance Liquid Chromatography/Mass Spectrometry(HPLC/MS). Analysis with HPLC/MS allows for customizable mobile phases, gradients, columns, and ionization to ensure the complete detection of OGSR. Using a Shimadzu Ultra Performance Liquid Chromatography (UPLC) coupled with an AB Sciex Q-Trap 4000 MS/MS, a method is optimized for the detection of Diphenylamine (DPA), Nitroglycerin (NG), and Ethyl Centralite (EC). The next steps for experimentation are summarized and include an elution study and a time-course study.

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LIST OF ABBREVIATIONS

AAS.....Atomic Absorption Spectroscopy

APCI .....Atmospheric Pressure Chemical Ionization

ASTM ..... American Society for Testing and Materials

CAD .....Collision Gas

CDC .....Centers for Disease Control and Prevention

CI.....Chemical Ionization

CID.....Collision Induced Dissociation

CUR ..... Curtain Gas

DBT..... Dibutyl Phthalate

DPA.....Diphenylamine

EC .....Ethyl Centralite

EDX ..... Energy Dispersive X-Ray

EI.....Electron Impact

ESI.....Electrospray Ionization

GC..... Gas Chromatography

GS1 .....Ion Source Gas 1

GS2 .....Ion Source Gas 2

GSR.....Gunshot Residue

HPLC ..... High Performance Liquid Chromatography

IGSR .....Inorganic Gunshot Residue

IMS .....Ion Mobility Spectrometry

IS ..... IonSpray Voltage

LC ..... Liquid Chromatography

MC ..... Methyl Centralite

MRM ..... Multiple Reaction Monitoring

MS ..... Mass Spectrometry

NAA ..... Neutron Activation Analysis

NC ..... Nitrocellulose

NG ..... Nitroglycerin

NQ ..... Nitroguanidine

OGSR ..... Organic Gunshot Residue

PTFE ..... Polytetrafluorethylene

SEM ..... Scanning Electron Microscope

SPE ..... Solid Phase Extraction

SPME ..... Solid Phase Microextraction

TEA ..... Thermal Energy Analysis

TEM ..... Temperature

TLC ..... Thin Layer Chromatography

UPLC ..... Ultra Performance Liquid Chromatography

V ..... Voltage

VOC ..... Volatile Organic Compound

2,4-DNT 2,4-Dinitrotoluene

1.0 INTRODUCTION

Gunshot residue (GSR) is the overarching title for the mixture of inorganic and organic particles that are produced when a firearm is discharged. (2) The residue consists of burnt and unburnt particles which originates from the propellant, primer, and other parts of the firearm or ammunition. When fired, the particles escape from openings in the firearm and can attach themselves to the shooter and objects in the vicinity. (5) Inorganic residues consist of vaporous deposits of metal salts that are detected as microscopic pieces of metal generated primarily from the ammunition primer: lead, barium or antimony. On the other hand, the majority of the organic residue originates from the propellant, with a lesser portion originating from the primer. Decomposition products from nitrocellulose (NC) and nitroglycerin, as well as stabilizers and plasticizers such as diphenylamine, ethyl or methyl centralite (MC), and dibutyl phthalate (DBT) are evaluated for the presence of organic gunshot residue. (3)

1.1 Ammunition

To understand how the detection and analysis of inorganic and organic GSR are completed, it is essential to understand how a cartridge of small arms ammunition is constructed and discharged. An unfired round of ammunition, or cartridge, is composed of four parts: primer, propellant, a projectile, and a cartridge case. To initiate discharge, the trigger is pulled thus releasing the firing pin. The firing pin hits the primer cap, which initiates the shock-sensitive primer. The flame produced by the primer then ignites the propellant. Ignition of the propellant causes a rapid decomposition, which produces a large amount of gas and pressure within the cartridge case. Taking the path of least

resistance, the pressure escapes its confinement by pushing the projectile out of the cartridge case and down the barrel of the gun. Each part of the ammunition must work effectively to guarantee the proper discharge of the projectile. (6)

1.1.1 Primer

Modern ammunition primer consists of three main components; lead styphnate, antimony sulfide, and barium nitrate. This combination was originally produced in 1921 and has remained relatively unchanged since then. (7) Lead styphnate is the shock-sensitive compound that sparks when primer cup is struck by the firing pin. Antimony sulfide acts as the fuel of the mixture while barium nitrate acts as the oxidizer. (6) A proper fuel to oxygen ratio combined with a sufficient spark ignites the compounds contained in the primer cup.

1.1.2 Propellant

The energy and flame caused by the decomposition of the primer provide sufficient energy to activate the next step of the discharge process, ignition of the propellant. Once ignited, the propellant rapidly decomposes and produces a large amount of energy, heat, and gas. The pressure build-up pushes the projectile out of the barrel of the gun, along with a significant amount of the gas produced during decomposition. (6) As firearms have developed throughout history, the use of propellants has adapted alongside them.

Black powder was the original propellant used in ammunition. Charcoal, Sulfur, and Potassium Nitrate are the primary compounds of black powder. (8) Black powder is

extremely corrosive and creates a large cloud of smoke when ignited. To mitigate these downfalls of black powder, modern smokeless powders were invented. (6) Modern smokeless powders are composed of varying amounts of nitroglycerin, nitrocellulose, diphenylamine, methyl centralite, ethyl centralite, and a myriad of other components. According to Goudsmits et al., there are upwards of 136 different components associated with smokeless powder. (9) Nitrocellulose, nitroglycerin, and other nitro- containing compounds provide the explosive nature of the smokeless powder mixture, which are combined with stabilizers and plasticizers such as DPA and DBT.

1.1.3 Projectile

For the firearm to perform as expected, the ammunition must also be equipped with an effective projectile. Efficient projectiles must be heavy enough to maintain their velocity and be a shape that is conducive for aerodynamic travel. Some of the first projectiles were a rounded stone or lightweight metal, like iron. These round metal or stone projectiles functioned as cannon projectiles, but the scaled-down version were not heavy enough to maintain stability after being fired from a firearm. Lead took the place of iron because of its greater density, ease of casting, and its lower cost. (10) As projectiles continued to evolve, metal alloys like brass and copper were added to lead bullets. This helped reduce some of the lead fouling in the barrel. Though most GSR originates from the primer and propellant, small amounts can come from the projectile and barrel during discharge.

1.1.4 Cartridge Case

The cartridge case houses and protects all other ammunition components during storage, transit, and use. The casing is often made of brass because of its ability to handle the high heats produced during firing. (10) Steel can also be used when brass is not available, as it can also withstand high heat and pressure. Assembly of the ammunition is as follows; prime the case, charge with propellant, insert the projectile, and crimp or seal the casing to allow for controlled gas escape. Crimping involves folding or bending the mouth of the casing to grip the inserted projectile. Once all four steps are complete, the cartridge can be inspected and then used. (6)

Figure 1 shows the cross section of a round of small arms ammunition. It labels several constituents of the ammunition, including all aforementioned components.

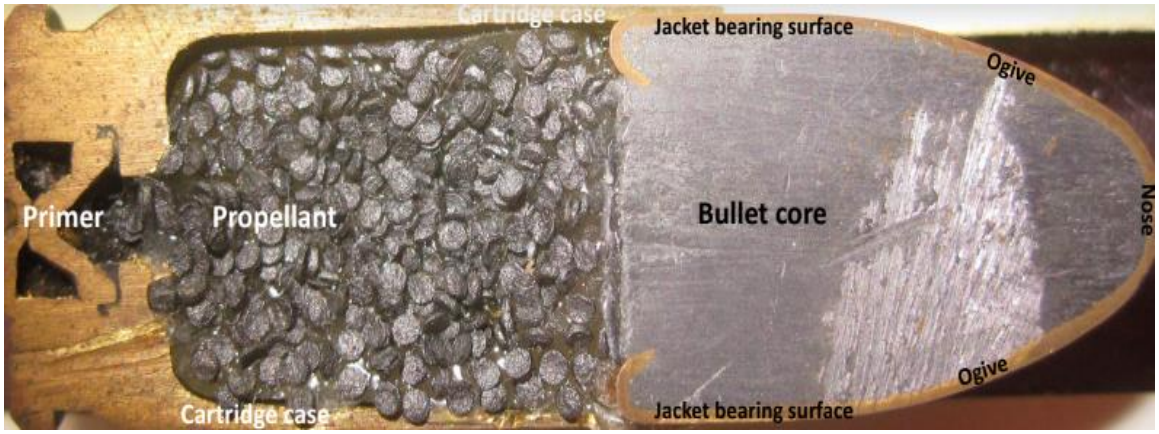

Figure 1. Cross Section of a Small Arms Ammunition

Photograph provided by Dr. Peter Diaczuk

1.2 History of Testing

Prior to the invention and widespread use of the Scanning Electron Microscope (SEM) in forensic laboratories, GSR was analyzed using a color test that detected nitrates. Theodoro Gonzales of the Mexico City Police proposed testing shooters hands for post-blast nitrates and nitrites with this color test. (1) To accomplish that, the shooter's hand would be dipped in paraffin and a mold of the hand is made**.** Once the paraffin sets, the hand was removed to form a cast. It was theorized that the post-blast residue would have stuck to the paraffin cast. The cast of the hand would be analyzed with the proposed color test. Diphenylamine in sulfuric acid solution reacts with the post-blast nitrates and nitrites, producing a color. That color change suggested the presence of gunshot residue. (11) Results from the dermal nitrate/paraffin test were controversial due to the test's lack of specificity. False positives could occur if that suspect or cast came in contact with chlorates, permanganates, iodates, and some metal oxides. Other false positives could occur from these more common household products; tobacco, fertilizers, some pharmaceuticals, fingernail polish, and dyes. Due to the environmental contaminants and lack of sensitivity this test was deemed unreliable and therefore is no longer used in current post-discharge analysis.

Gunshot residue shifted from non-confirmatory color tests to confirmatory detection of the primer's heavy metals in the early 1960s. (1) Analysis of inorganic GSR was completed on a wide variety of instrumentation. Neutron Activation Analysis (NAA) has the capability to test a wide range of metals at trace levels, including those that compose inorganic GSR. Along with IGSR analysis, it was used for distance

determination. Though theorized that NAA could analyze inorganic GSR residue, lead cannot be analyzed without the use of a nuclear reactor. Due to this stipulation, NAA is not a widely used technique for GSR testing. Another possible technique is flameless Atomic Absorption Spectroscopy (AAS). Flameless AAS is preferable to NAA because of its ability to detect lead, barium, and antimony. Though successful in extracting and detecting lead and barium, its inability to successfully extract antimony from a collection swab prevents the implementation of AAS into crime laboratories. Analysis of IGSR using AAS results in a large number of false negatives, which supports its inadequacy. Other methods, including Inductively Coupled Plasma (ICP) iterations, can be used for inorganic GSR analysis. However, each method has its respective issues that has prevented their implementation in crime labs. (7) In 1974, a Scanning Electron Microscope coupled with energy dispersive x-ray (EDX) became available for inorganic GSR analysis. SEM/EDX became the most frequently used technique for the analysis of inorganic GSR. (12)

SEM/EDX is the most common technique for inorganic GSR analysis due to its ability to provide morphological images and chemical compositions of individual particles. (13) SEM/EDX's has the ability to discriminate one primer residue particle a part from thousands of other particles that are collected on the stub. (14) According to American Society for Testing and Materials (ASTM) guidelines, a particle that is characteristic of inorganic gunshot residue has an average diameter of 1 micrometer and must contain lead, barium, or antimony. SEM/EDX can obtain this information in a non-destructive manner, which is advantageous if the sample is small or needs to be re-run.

Though it has been heavily researched and is the most widely used method in testing GSR, SEM/EDX does not come without its faults.

The compounds found in inorganic GSR are not exclusive to the primer. Brake lining, fireworks, paints, and cartridge-operating occupations produce particles that are similar to primer particles. Exposure to these environmental and occupation sources could lead to false negatives. (12) In general, particle evidence has a transitory nature. Particles can easily be transferred from person to person, or easily come off of the suspect. Therefore, false positives can occur if a bystander came in contact with an object or person that was originally near the shooting. False negatives can occur if the particles did not effectively adhere to the shooter. Particles can also detach from the shooter from daily activity, like hand washing. (4) Particulates have even been found to transfer from shooting hand to non-shooting hand after wiping hands with a paper towel. (11)

Additional complications resulting in false-negatives are caused by the increased use of lead and heavy metal-free ammunition. (12) Concerns of lead toxicity have caused the beginnings of an ammunition primer reform; bans of lead shot for hunting have been implemented, in addition to manufacturing companies removing lead from some of their products. Leadless primers would remove one of the three markers indicative of a positive GSR sample. (15) Elements that could appear in non-lead based ammunition could include zinc, tin, copper, aluminum, and sodium. (7)

ASTM verifies that detection of all three elements is considered characteristic of firearm discharge and not naturally occurring, while the detection of two out of three elements is consistent with GSR. (15) Removing lead from ammunition would force

guidelines to include a wider range of metals. Changing the composition of the primer does not significantly change the composition of organic gunshot residue. Therefore, as ammunition changes to lead and heavy metals free, OGSR testing would not be affected in the same way as IGSR testing. (5) Due to the transition into the heavy metal free primer and the other aforementioned issues, organic gunshot residue has become a novel topic of research and has possible implementations in crime laboratories.

1.3 Organic Gunshot Residue

Organic gunshot residue results from the burning of propellant during the firing process, as well as remaining unburnt particles. Most of the particles and the gas produced are also ejected with the projectile out the barrel of the firearm, but both the gas and particles are small enough to escape any other openings in the firearm. The deposit of gas and particles on the shooter's hands can potentially connect them to the shooting of a firearm. (3,9)

In 1978, Mach et al. defined ethyl centralite, 2,4-dinitrotoluene (2,4-DNT), and diphenylamine as the three most characteristic OGSR components. (16) Since then the discussion about the composition of organic gunshot residues has continued to evolve. Modern smokeless powders contain the original characteristic OGSR compounds but nitrocellulose, nitroglycerin, or nitroguanidine (NQ) are now a part of the characteristic components. (5) In addition to the nitro-containing base compounds, modern smokeless powders contain flash suppressants, stabilizers and plasticizers. DPA, EC, and MC frequently top the list when discussing the most common additives in smokeless powder.

DPA is the most common stabilizer. EC and MC are the most popular compounds to gelatinize the NG. (13) DBT is a commonly used plasticizer to allow the smokeless powder to be manufactured without cracking or breaking. There have been over 130 organic compounds listed as possible contributors to smokeless powder and OGSR. (2) The chemical structures of NG, DPA, EC, and DBT are shown in Figure 2.

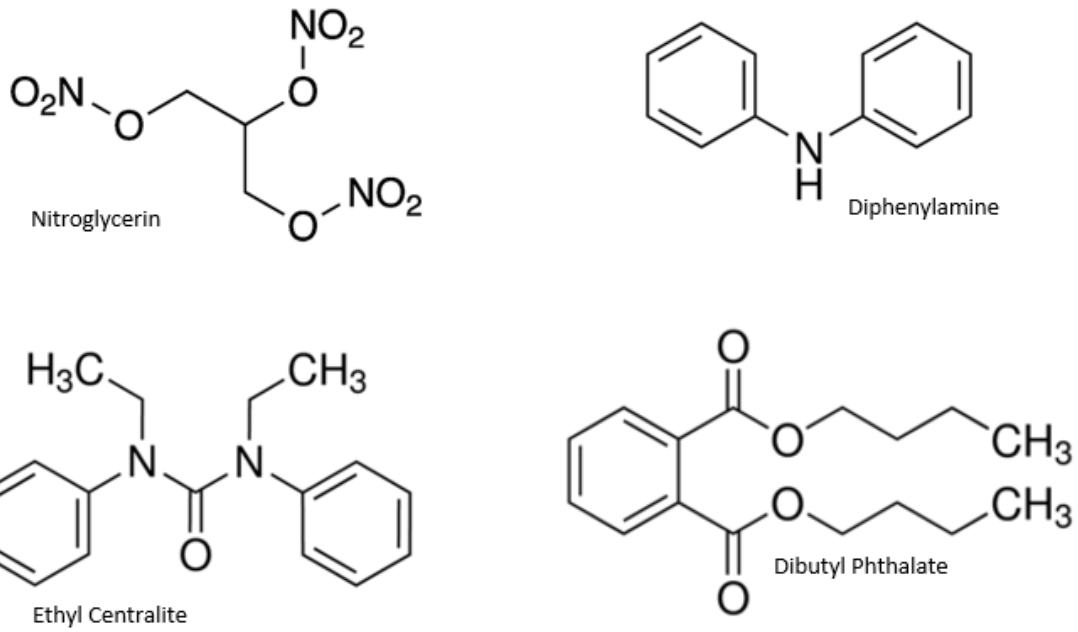

Figure 2. Structures of OGSR Compounds

1.4 History of OGSR Analysis

OGSR analysis has not always used advanced confirmatory instrumentation as is does today. In the 1960s analysis of OGSR was primarily completed by Thin Layer Chromatography (TLC). (2) TLC is a simple and rapid approach to detection. A major

disadvantage to TLC is that each compound must be extracted and prepared separately**.** Other disadvantages of TLC include the inability to quantitate compounds of interest, analysis consuming a large portion of the sample, and the time-consuming nature of visualizing results. Due to these drawbacks, the use of TLC to analyze OGSR was discontinued.(11)

In the 1970s, the feasibility of using gas chromatography to analyze smokeless powder was researched. (2) Gas chromatography can separate complex mixtures. It's compatibility with various detectors like flame-ionization, ion mobility spectrometry, and thermal energy analysis adds to its versatility. Each detector has its own set of advantages and disadvantages. When a proper detector is coupled with gas chromatography, the technique proves to be a rapid, highly selective method that is able to detect smokeless powder components in samples as small as 10ng. (7,11) Another advantages is its ability to be easily interfaced with Solid Phase Micro Extraction (SPME). Directly injecting SPME into the Gas Chromatograph (GC) eliminates the need for complex or time-consuming sample preparation. Additionally, SPME-GC has the ability to accurately qualify and quantify the various components of smokeless powder. Though a widely used and documented method, the high heats used in the GC ovens and in the injection port are not compatible with several of the compounds characteristic of OGSR. (17) Nitrocellulose, nitroglycerin and various stabilizers are examples of compounds that cannot be detected through GC due to thermal degradation. Gas chromatography was the primary technique used from 1970 to 2000 and is useful technique for thermally stable compounds. However, its major flaw is its inability to detect NG or NC, which turned

researchers to other techniques including IMS (Ion Mobility Spectroscopy) and LC/MS (Liquid Chromatography/Mass Spectrometry). (2)

The 2000s brought about smokeless powder analysis with IMS and coupling SPME with IMS. The widely used technique is one of the most sensitive and robust methods in the analysis of explosives. (2) IMS is a portable instrument that uses atmospheric pressure chemical ionization (APCI) to presumptively detect explosives. In comparison to GC-thermal energy analysis (TEA), IMS provides more sensitivity and selectivity while maintaining a rapid response time. When IMS is coupled with SPME, this technique can be used to detect the odor signatures of previously identified explosive compounds. (18) Coupling these techniques enhances the level of detection when compared to using IMS alone. Unlike other techniques, SPME-IMS has the capability to detect nitroglycerin and nitrocellulose. IMS can only be used as a screening tool because it does not provide structural information about the compounds detected. (2)

In the 2010s, HPLC coupled with mass spectrometry and UPLC coupled with tandem mass spectrometry was introduced to OGSR analysis. One advantage of using liquid chromatography coupled with mass spectrometry is its ability to successfully separate OGSR compounds rapidly while maintaining a high resolution. (19) Unlike gas chromatography, liquid chromatography operates at low temperatures and therefore does not cause the thermal degradation of compounds like NG and NC. Mobile phases can be customized with additives such as ammonium acetate or acetic acid to enhance the ionization of OGSR compounds. (20) Enhancement of the compounds allows for better detection of the compounds of interest. Some mass spectrometers have the ability to

switch their ionization mode from negative to positive, and vice versa, during analysis. (19) Liquid chromatography analysis has many advantages but also has its shortcomings. To ensure the longevity of the instrumentation, copious amounts of sample preparation must be completed on the samples. Sample preparation and mobile phases use large quantities of solvents. Disadvantages of HPLC are unrelated to the stability of compounds being detected, and therefore are preferable over other methods.

1.5 Why OGSR over ISGR?

Using SEM/EDX has been included in forensic science laboratory's standard operating procedures since the early 1970s. (12) Transitioning from a heavily documented and reliable method to a more novel procedure would require the newer method to have significant benefits. Some benefits of choosing OGSR over IGSR analysis include organic residue being less transitory, a higher persistence on hands, and a lesser likelihood of finding all the compounds characteristic of smokeless powder in an environmental or occupational source. (3,9)

DPA, EC, and many of the other compounds found in OGSR are lipophilic, meaning tending to adhere to lipids. In the anatomy of human skin, there are various types of lipids where OGSR compounds could adhere. In the first layer of the epidermis, there is a lipid bilayer horizontally separating two corneodesmosome layers. Intercellular lipids are also found between the various types of skin cells. Therefore, after a firearm is discharged, the organic vapor settles on the skin and starts to permeate. As it permeates, it adheres to the various lipids. Due to the organic residues adhering to the skin, the

compounds of interest are less likely to be brushed or wiped off. The lipophilicity of the compounds reduces the number of false positives because objects that come in contact with a shooter's hands are less likely to pick them up compared to inorganic particles. The nature of the residue also provides fewer false negatives due to compounds more readily adhering to the skin. (4)

Detecting inorganic gunshot residue on the shooter's hands is improbable after as little as two hours post-shooting. (3) Simple and routine activities such as hand washing, drying hands with paper towels, wiping hands on clothing, and putting hands in pockets have been shown to remove inorganic particulates from hands. (11) Due to the aforementioned lipophilicity, a proper method could be developed to detect OGSR twenty-four hours after firing with the advantage of targeting multiple compounds. (4)

Finally, there are fewer environmental or occupational occurrences of detecting OGSR as a mixture. Centralites, NG, NC, and DPA can be found as singular compounds but there are limited instances where they are found simultaneously. (13) Nitroglycerin is often found alone in medical and pharmaceutical environments. Nitroglycerin can be commonly found in oral or transdermal pharmaceuticals to treat certain heart conditions. (21) Nitrocellulose, a primary component in single-base propellants, can also be located in varnishes, lacquers, printing, and pharmaceuticals. Its hydrolysis after discharge makes it difficult to distinguish NC from propellants and NC from occupational occurrences. (13) Size exclusion chromatography can be implemented to determine the difference between the propellant and occupational nitrocellulose if needed. (11) Centralites are infrequently found in environmental or occupational environments; they are primarily

used in propellants and ammunition. DPA can be encountered in the food industry, explosives, dyes, soil, and groundwater. DPA uniquely reacts with the decomposing products of NG and NC. As a result of this, environmental contaminants have not caused false positives. (9) There are many occupational or environmental opportunities for these components to arise and be detected as a singular compound, but finding them as a combination has been limited to only detection of organic gunshot residue.

1.6 High Performance Liquid Chromatography Theory

One of the analytical challenges presented in forensic science is the need to identify and quantify compounds of interest. For this to be done effectively, the components need to be relatively pure. (22) This can be done with a myriad of techniques but is often completed with HPLC coupled with MS. Separation of complex mixtures is done by using chromatographic instruments such as HPLC. To confirm the structure of the separated components, a detector with the capability to provide structural information, must be present. For example, a mass spectrometer is a detector that has the ability to provide structural information. (23)

Columns pre 1960s were composed of primarily porous silica or alumina absorbents, which resulted in a relatively polar stationary phase. When paired with a relatively non-polar stationary phase, ‘normal- phase’ HPLC separation was born. In the late 1960s, the porous silica or alumina-based beads were reacted with n-C18 molecules, which created a non-polar stationary phase. Paired with polar mobile phases, ‘reverse-phase’ separation was created. Aqueous mobile phases modified with polar solvents give

‘reverse phase’ more versatility than its counterpart. Strong molecular forces in ‘normal phase’ is caused by adsorption. Partitioning is the mechanism that separates compounds in ‘reverse phase’ chromatography, which relies on much weaker intermolecular bonds. These weaker intermolecular bonds lead to lower equilibration times and ease of regeneration. (23)

Chromatography separates compounds based on their affinities to both the mobile phase and the stationary phase. In the case of liquid chromatography, the mobile phases are solvents or mixtures of solvents, and the stationary phase is coated onto beads that are tightly packed in stainless steel columns. A sample is loaded onto the instrument and the mobile phase carries it through the column and to the detector. The sample is introduced to the stationary phase in the column. There the mixture will separate into individual components based on their affinity to the stationary phase. Compounds with less affinity to the stationary phase will travel more rapidly out of the column compared to compounds with greater affinity. The amount of time a compound spends on a column is described as retention time. Retention times of the unknown compound cannot be used as a confirmatory tool but can be compared to libraries as a presumptive identification. (22) Figure 3 shows the basic structure of a liquid chromatography instrument.

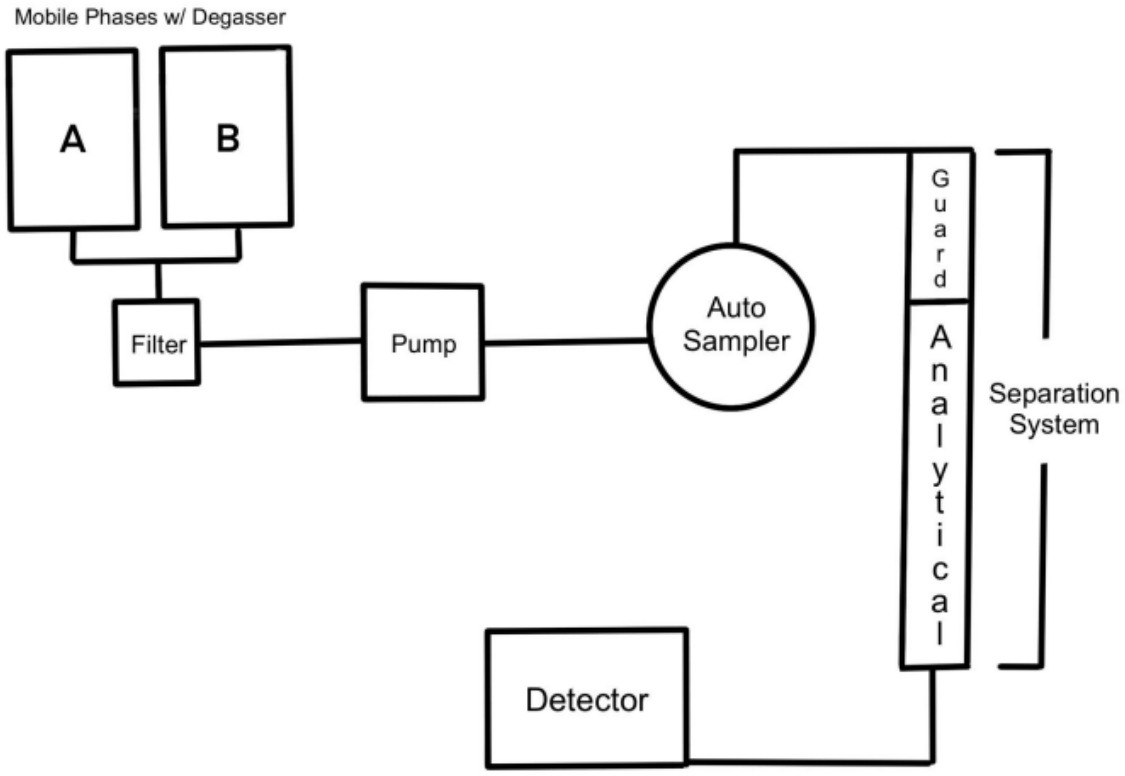

Figure 3. Basic Construction of an HPLC

1.7 Mass Spectrometer Theory

Mass spectrometers are comprised of three main components; an ion source, a mass analyzer, and a detector. Samples can either come from the attached chromatographic instrument such as a GC or an LC or be directly injected through the inlet. The ion source ionizes the compounds and then the ions move to the mass analyzer, which monitors the compounds. Exiting the mass analyzer, the ions enter the detector which produces a mass spectrum. The mass spectra are then saved in the attached data

system. The ion source, mass analyzer, and detector are all under vacuum. (24) Figure 4 shows a generic set up of a mass spectrometer.

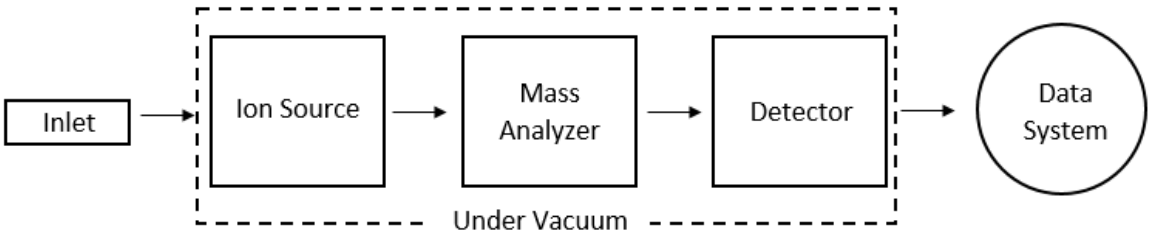

Figure 4. Basic Construction of a Mass Spectrometer

Electron impact (EI) and chemical ionization (CI) are the two most common types of ionization techniques. Electron impact is a hard ionization technique that causes high fragmentation. But because of the high fragmentation, the molecular ion is not always detected. CI is a soft ionization technique that can detect the molecular ion in positive or negative mode. The addition of the reagent gas converts the hard EI technique to a softer CI technique. Both techniques interface well to gas chromatography. (23)

Electrospray ionization (ESI) and atmospheric pressure chemical ionization are both softer forms of ionization and interface well to liquid chromatography. ESI uses an electric field and a gas stream to aid in the ionization of compounds. The molecular ion often occurs, with little fragmentation. Nebulizer gas and a corona discharge needle are used in APCI to ionize compounds. APCI is chemical ionization but under atmospheric pressure. (23)

Ions travel from the ion source into the mass analyzer. In a quadrupole mass analyzer, there are four poles; two with radiofrequency and two with direct current voltage. Single mass to charge ions can travel through the quadrupole, and only specifically selected ions will make it through to the detector. All of the other ions will be deflected to the poles and will not be detected. (24)

Tandem MS is the linking of multiple quadrupoles to produce an improved fragmentation of compounds. In a triple quad; Q1 is the first mass analyzer, Q2 is the collision cell, and Q3 is the third mass analyzer region. Q1 sorts the initial ions based on their mass to charge ratio, and only allows selected ions to travel to Q2. The single ions that are selected from Q1 are also called parent ions. In Q2 the ions are subjected to a collision gas or Collision induced dissociation (CID), which is a fragmentation technique that uses an electrical charge to accelerate the ionized compounds into neutral gas molecules. Q3 can be an ion trap or a second quadrupole. It can scan all ions produced or select specific ions. The ions that travel through Q3 are called product ions. (23)

2.0 PUBLISHED STUDIES

2.1 OGSR sampling

The goal of OGSR sampling is to collect as much of the compounds of interest from of the sampling surface, as possible. Similar to IGSR, this can be completed using various sampling techniques. These techniques include gel lifts, nasal collection, collecting GSR off of hair, vacuuming, swabbing, and tape lifting. (7) The three techniques often used are vacuuming, swabbing, and tape lifting.

2.1.1 Vacuuming

Vacuum lifting collects GSR particles from clothing and other porous materials and then concentrates them on a filter disk. (25) Vacuuming uses a filter to pull air and particles through its system. Although vacuum lifting typically reserved for inorganic sampling, it can also be used for sampling organic residue. (2) As the air and particles are being pulled through the system, filter(s) will trap any particles that are too large to pass through. Different pore sizes will trap different sized particles. When solely sampling inorganic gunshot residue, larger pore sized filters, like a 20µm filter, are installed to trap the larger inorganic particles. When solely sampling organic residue, smaller pore sized filters, like 0.8µm filter, collect any organic particles. If sampling both IGSR and OGSR, the larger pored filters are first, followed by a smaller pored filter. This allows the inorganic particles to be trapped while the organic particles travel through, and onto the next filter. (7) After sampling is completed, the filters are removed and prepared for analysis. The filters trapping IGSR are stubbed using an adhesive carbon stub suitable for SEM examination. (26) Filters containing organic residue are placed into an elution

solvent to remove any particles from the filter. After elution is complete, the sample can be analyzed with various instrumental techniques such as HPLC or GCMS. (7)

When sampling IGSR with vacuum lifting, the experimenters must take extra precautions with the clothing and gloves they are wearing. All clothing must be devoid of IGSR or other small metal particles. (26) As mentioned in the introduction, the particles that comprise inorganic gunshot residue are incredibly transient. Contamination, leading to a possible false positive, is possible unless analysts are being incredibly careful and using extra precautions.

Vacuum lifting can quickly cover a large sample surface. With its filtration system, this sampling technique pre-concentrates the residue onto the filter. (26) However, a large drawback of vacuuming is the inability to safely sample human skin. If presented with a suspect who needs their hands or face sampled, more than one sampling technique would have to be implemented. (25)

2.1.2 Tape Lifts

Tape lifting is the most common technique to sample IGSR from hands. (25) Like vacuuming, tape lifting can be implemented to collect OGSR but is not the most common. (2,12) Sampling with tape involves placing the adhesive side of the tape on the sampling surface and removing it multiple times until it is no longer tacky. The entire surface must come in contact with the adhesive to ensure the it is properly sampled. After the sampling is completed, the preparation of the tape for analysis depends on the style of tape as well as which residue will be targeted.

Multiple types of tape lifts can be used to sample gunshot residue. Metal stubs coated with a carbon adhesive are commonly used for IGSR collection but can be modified to sample OGSR as well. (5) Carbon adhesive tape, double-sided tape, 3M poster tape, and polytetrafluorethylene (PTFE) have all been studied. (27)

Though more successful in recovering IGSR than swabbing, tape lifts are also more likely to pick up skin cells and unwanted fibers. (7,27) Picking up skin cells and fibers on the tape lift will reduce the tackiness, and therefore particles could be missed. (27) Unlike vacuuming, tape lifting will only sample particles on the surface and is safe to use on human hands and faces. (2) Gassner et al. compared the efficacy of tape to swabs and found that tape was more successful at sampling OGSR than both cotton and polyester swabs. Benito et al. found that PTFE tape was capable of recovering OGSR but was more successful when analytes were spiked onto the surface. (5,27) The problem is that neither of the researchers covered the necessity to carbon coat tape/stubs before SEM/EDX analysis and the deleterious effects that carbon has on organic residue.

Carbon-based adhesives are conducive for moving directly from sampling to analysis on the SEM/EDX, but non-carbon based tapes must be carbon coated before analysis. (5) The carbon coat does not interfere with the detection of inorganic residue but significantly limits the detection of organic residue. (2) Therefore, if a non-carbon-based tape was used for sampling, half would have to be uncoated to have the chance to successfully detect both. This is possible by covering half of the stub with a layer of parafilm and PTFE before sampling. Once sampling is completed, the PTFE is removed and placed in an elution solvent. The parafilm is removed and discarded**.** The stub can

then be carbon coated for IGSR analysis. (5) Figure 5 illustrates how a stub is prepped for both OGSR and IGSR collection.

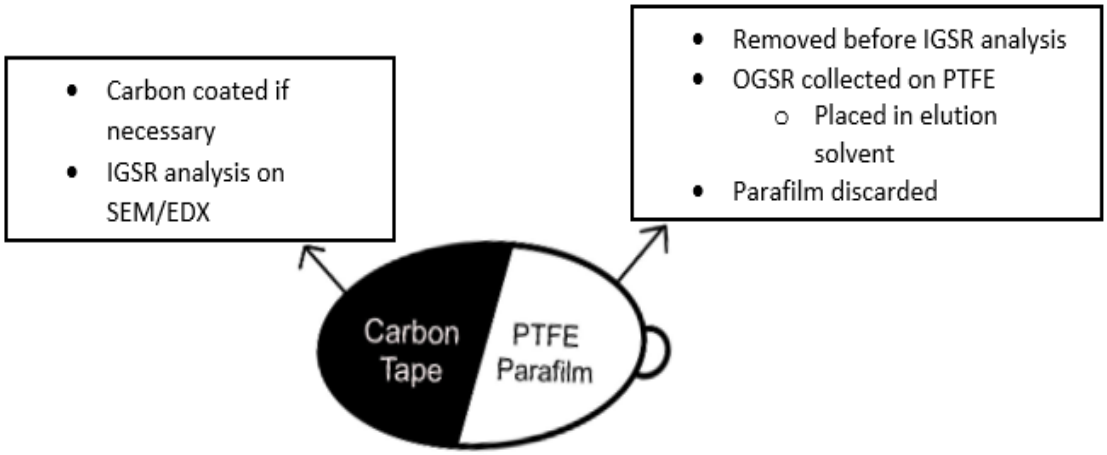

Figure 5. Example of Carbon Tape with PTFE

2.1.3 Swabbing

Swabbing is the most commonly used technique to sample organic gunshot residue, but it has the potential to collect both IGSR and OGSR. (2,11) Swabs, which are typically moistened with a solvent, are repeatedly wiped on the sampling surface. The solvent must be suitable for the collection of OGSR and choosing a proper solvent is discussed in the section below. Swabs can be comprised of cotton, polyester, wool, filter paper, or is a medical wipe. (11) After sampling, the compounds can be eluted from the swab by use of an elution solvent, or by placing the swab in a vial and collecting the volatile organic compounds (VOC) using solid phase microextraction. (11,28) Cotton swabs, polyester swabs, and medical wipes are the three swab compositions most discussed in the literature. Overall, there is no agreement on the most successful technique.

Alcohol wipes have the possibility to pick up both inorganic and organic residue. When sampling explosive residue from non-porous surfaces, alcohol wipes recovered slightly more residue than the cotton and polyester swabs. When sampling inorganic explosive residue, the alcohol wipe recovered more residue than cotton swabs but less than polyester. (29) Taudte et al. compared the recovery of alcohol wipes to tape lifts and found that more than an 80% recovery of OGSR was possible when paired with the proper elution solvent. (12) Zhang explained the composition of alcohol wipes and discussed how they were made for particle recovery with limited contamination. From Zhang's research, Bell used alcohol wipes in skin permeation studies. (4,30)

Gassner and Weyermann tested polyester and cotton swabs to determine whether the swabs individually interfered with sampling. When using a C18 column, polyester swabs contributed a minor peak directly before the DPA peak, while cotton swabs did not. Though the peak was present, it did not interfere with the detection of DPA. (27) Polyester produced over 80% recovery of all desired compounds, including DPA and EC, and collected more than the cotton swabs. It was theorized that the weave of the polyester is more conducive to picking up GSR than cotton, resulting in the larger recovery. Although these studies showed positive results for using polyester swabs, they also showed inconsistent results when the person swabbing changed. Some experimenters had a high recovery while others had quite low. (31) Gassner and Weyermann compared polyester swabs to tape and found that tape recovered more residue than the polyester swabs. A major advantage that swabs have over tape is the lack of adhesive; there is less

sample prep needed with swabs because the adhesive does not dissolve in the elution solvent. (31)

Cotton swabs produced similar recovery to both wipes and polyester swabs, but are more cost-effective. (29,32) In the same study that looked at polyester swabs, the recovery of OGSR from cotton swabs was also above 80%. Further studies have shown that cotton swabs are effective for the recovery of nitroglycerin and OGSR. (7,11) Swabbing is effective for sampling OGSR and IGSR. Using a method similar to vacuum filtration, different filters will collect different residues based on size. The steps that differ from vacuuming is the addition of swabbing the hand and then removing the compounds of interest on the swab in elution solvent. The elution solvent is then transferred into a vial that can connect to filters. The elution solvent flows through the filters, and the residues get trapped in their respectively sized filters. (12) The respective filters will be collected and analyzed by either an SEM/EDX or chromatography instrumentation. Figure 6 shows the method of using swabs and various filters to collect both IGSR and OGSR.

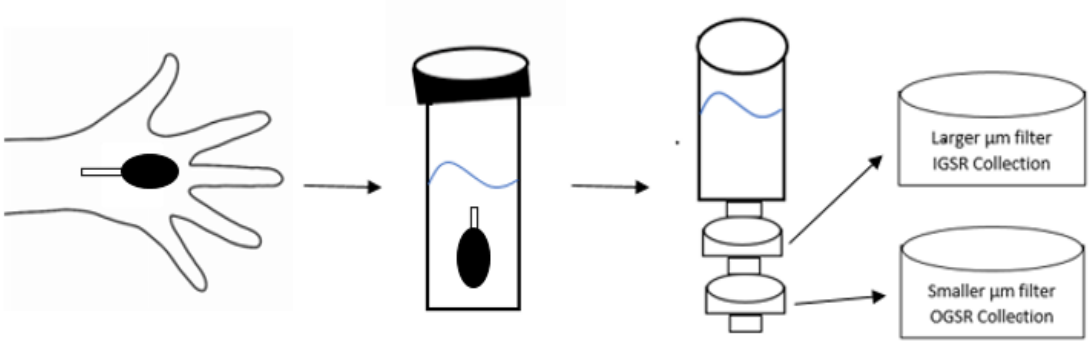

Figure 6. Mechanism of Recovering OGSR and IGSR using a Swab.

2.1.4 Swabbing Solvent

When using a swab to sample OGSR, the swab must be moistened with a solvent conducive to the swab and the residue. An aqueous or organic based solvent is often used to moisten the swab. Desired characteristics in a solvent include high recovery of compounds of interest, minimal interference with compounds of interest, safety to the person sampling, and safety to the surface of what is being sampled. The likelihood of a perfect solvent for sampling OGSR is slim, but a balance of the aforementioned characteristics is what leads to the solvent choice. Methanol, ethanol, isopropanol, acetone, and water are five solvents commonly discussed and compared in the literature. (2,7,12,33)

Minimal interference and maximum recovery for compounds of interest are critical for choosing a swabbing solvent. Research into the use of organic solvents for recovery of OGSR, like acetone, isopropanol, methanol or ethanol, and aqueous solvents, such as water, have been well documented. For example, water has been proven to lead to high recovery of nitroglycerin. This success however, is negated by microbial growth in the aqueous solvents, which leads to the degradation of NG. (33) Organic residue from explosives or smokeless powder are readily soluble into organic solvents, leading to high recovery. Organic solvents can also readily dissolve and collect other compounds when sampling, including foreign and unwanted compounds from the sampling surface. (7) Extra steps, such as solid phase extraction (SPE), are necessary to rid the sample of the unwanted compounds that interfere with the desired compounds. (7,34)

Human skin, both hands and face, are subjected to sampling techniques. Therefore, the sampling technique must not cause excess adverse effects to the skin. Water, isopropanol, ethanol, methanol, and acetone are often used in OGSR sampling. (3,5,13,28) Water is the only solvent that will not cause any adverse reactions. Acetone, isopropanol, and ethanol are often components or active ingredients of products that people use in their daily lives. (31) Isopropanol is often used as a disinfectant. (35) Acetone is used in nail polish removers and other commercially available paint removers. (36) Ethanol can be found in beauty products. Exposure to isopropanol, acetone, or ethanol even in small doses, would cause mild irritation and dryness. Methanol is not often found in beauty products because of its toxic effects on the human body. If exposed to methanol, the Centers for Disease Control and Prevention (CDC) suggests removing oneself from the exposure and immediately seeking medical attention. Though the small amount of solvent used in sampling OGSR is unlikely to cause methanol poisoning, exposure should be prevented. (37)

No solvent perfectly fulfills all qualities desired in a sampling solvent. Water needs extra drying time and can degrade nitroglycerin. Organic solvents require additional steps in extraction because they collect compounds that cause interference during sampling. The only definite answer provided by the literature is methanol, though it should not be used when sampling human skin. To determine which solvent would render the highest recovery in a specific experiment, a sampling study should be completed.

2.1.5 Elution Solvent

Regardless of the sampling technique, the residue must be removed and collected from the tape, swab, or vacuum filter so analysis can be completed. This is completed by a separate organic or aqueous solvent. Similar to sampling solvents, there is little continuity in which solvent performs the best. A proper elution solvent should remove the compounds from the sampling medium without causing interference. Removal is often aided by sonication, by centrifuging the sample, or by rocking the sample. Isopropanol, acetonitrile, methanol, acetone, ethanol, and water are commonly used elution solvents for explosives and gunshot residue. (2,14,31) Other solvents that can be used are hexane and cyclohexane. (4)

Various studies have found different solvents, or mixtures of solvents, to produce the highest recovery. For example, a study done by Zeichner and Eldar found that a combination of 80/20 water/ethanol produced the highest recovery. (38) A study by Ali et al. found that a combination of 40/40/20 methanol/acetone/acetonitrile produced the highest recovery for the six compounds that were analyzed. (14) Many studies did not complete a recovery study with various solvents but produced positive results with the solvents mentioned previously.

To obtain the highest recovery possible, the sample in the elution solvent must be sonicated or disrupted in some way. (38) Percent recovery has been tested with various sonication times ranging from five minutes to thirty-five minutes. The tests were completed at five-minute increments. (12,14) When comparing sonication between 5, 10, 15, and 20 minutes, Ali et al. found that there was a significant difference between 10 and

20 minutes, but no difference between 5, 10, and 15 minutes. Sonication for 20 minutes surprisingly decreased recovery of some explosives. (14) When comparing between 20 and 35 minutes, there was little difference in percent recovery for the two times. (14)

Each technique will need a unique sampling method, sampling solvent, and elution solvent. When determining which sampling and extraction technique should be used for a specific method, the surface that will be sampled, the compounds being detected, and the analytical methods need to be taken into consideration and optimized. (2)

2.2 Method Development for HPLC/MS

Reverse phase chromatography is common when analyzing explosives and organic gunshot residue. Using a binary system, an aqueous and an organic mobile phase are used to create a gradient. The gradient causes the separation of the compounds of interest. A small percentage of anion adducts, salts, or acid is added to the mobile phases to facilitate electrospray ionization. Examples include formic acid, chloride, ammonium acetate, ammonium formate, or nitrates. (20,39) In reverse phase, polar or slightly polar mobile phases are paired with a non-polar column. A C18 column or a C8 column are both examples of non-polar columns that are used in the analysis of OGSR. (13,40) Overall, the choice of mobile phases and the column depends on which compounds are being focused on for detection. After the mobile phase and the column are chosen, a gradient is optimized. A successful gradient has good separation and resolution of all compounds.

A mass spectrometer is often used as the detector attached to the chromatographic front end. The ionization technique is dependent on the instrument with which it is coupled. For example, if a gas chromatography/mass spectrometer is used, the ionization technique would most likely be electron impact or chemical ionization. If liquid chromatography is implemented, APCI or ESI would be used. (23) Each ionization technique has qualities that are beneficial to the detection of gunshot residue and qualities that are not.

EI is not a technique commonly implemented in the analysis of GSR or explosives. It has been used to successfully analyze the less thermally labile primers. High explosives and the components of smokeless powder are prone to fragmentation, therefore a hard ionization technique such as EI can prevent identification. CI is a softer technique and therefore a molecular ion can be more commonly identified. (19) Negative mode CI was found to be more sensitive than positive mode, and when temperatures were reduced it can detect high explosives. (41) Because these two techniques are coupled with gas chromatography, they are used frequently as the techniques that couple with liquid chromatography.

Electrospray ionization is a soft ionization technique that occurs under atmospheric pressure. ESI is frequently used in the detection of OGSR and high explosives. Positive ion mode is used to detect the stabilizers and additives, while negative mode detects the nitro containing compounds. Electrospray ionization detects nitrate esters when detecting high explosives, which is better than its counterpart, APCI. (19) Many authors have used ESI in their GSR or explosive related experiments. These

include Tong et al. who used positive mode to detect additives, and Mathis and McCord used negative mode to detect compounds like nitroglycerin. (39,42)

APCI is similar to ESI because it is a soft ionization technique that is conducive to liquid chromatography. (23) Both positive and negative ionization modes are used similar to ESI. Positive mode detects stabilizers and additives, while negative mode detects compounds with nitro groups. DeTata et al. found that using APCI in negative mode produced a greater response to nitro- containing compounds. APCI in negative mode was therefore the preferred choice. (40) Outside of nitro ester compounds, APCI has been found to perform better than ESI in ionizing GSR compounds. (19)

With newer instrumentation, there is the possibility of combining ESI and APCI. The combination minimizes the negatives and accentuates the positives of both techniques. Thomas et al. used an instrument from Waters™ that could switch from APCI to ESI and from positive mode to negative mode quickly. Switching from the different techniques and modes could also be completed without disruption from the method. This technique allowed for the highest sensitivity for each compound that was being detected. Smokeless powder additives, such as DPA, DBT, and EC, were ionized under ESI positive mode. Nitroglycerin was ionized under ESI negative, and APCI negative mode was used to ionize compounds like 2-Nitrotoluene and 2,4-Dinitrotoluene. Using this method, Thomas detected eighteen out of the twenty-one prechosen compounds. (43)

Like the sampling method, the instrumentation method used would depend on what compounds were chosen to be detected. The available instrumentation could also

guide which technique is used. If the instrumentation was only capable of a specific ionization technique, the method would have to be guided and optimized based on the capability of the available laboratory instrumentation.

3.0 MATERIALS AND METHODS

A Glock 9mm Luger Semi-Automatic Pistol was the firearm chosen for the detection and time course study. The firearm used was previously purchased by the Boston Police Department. During the experimentation, the Boston Police Department maintained possession of the firearm. A 9mm Luger, 115 grain full metal jacket GFA ArmsTec ammunition was paired with the firearm. (Natick, MA) The bullets were separated using a bullet puller. Experiments were carried out using a Shimadzu (Kyoto, Japan) Ultra Flow Liquid Chromatograph and an AB Sciex (Framingham, Massachusetts, U.S.A.) Q-Trap Electrospray Ionization Tandem Mass Spectrometry (ESI/MS/MS, SCIEX, Waltham, Massachusetts, U.S.A.). Sciex Analyst® (version 1.6.2) software was used to control the LC/MS/MS system. The Figure below shows the ammunition as it is intended to be used, and it deconstructed.

Figure 7: Cartridge (right) and Deconstructed Cartridge (left)

3.1 LC Optimization:

A Waters (Milford, Massachusetts, U.S.A) XBridge™ C18 (2.1x50m) 3.5-micron column was kept between 40°C and 80°C for analysis. Solvent A, HPLC grade water + Methanol (HPLC optima grade, 99.9% purity) + 0.1% Acetic Acid (Glacial reagent), and Solvent B, Acetonitrile (HPLC optima grade, 99.9% purity) + 10% Methanol (HPLC optima grade, 99.9% purity) + 0.1% Acetic Acid (Glacial reagent) were used under a binary pump system that flowed at 0.300mL per minute. Both pumps were conFigured to allow a maximum pressure of 1451 pounds per square inch. A 5µL injection volume was used for all samples.

Two gradient timetables are shown in Table 1. The timetable on the left shows the method that was used to detect all four compounds; EC, DPA, NG, and DBT. The timetable on the right shows the final method that only detects EC, DPA, and NG**.** DBT was removed from the method after contamination issues and is explained in another section.

Table 1. LC Gradient with DBT and Final Method without DBT

Method with DBT			**Final Method**		
Time (min)	**Solvent A (%)**	**Solvent B (%)**	**Time (min)**	**Solvent A (%)**	**Solvent B (%)**
0.00	70.00	30.00	0.00	70.00	30.00
1.00	55.00	45.00	1.00	55.00	45.00
3.00	55.00	45.00	3.00	55.00	45.00
6.50	50.00	50.00	6.50	50.00	50.00
7.50	50.00	50.00	7.50	50.00	50.00
9.50	45.00	55.00			
11.00	70.00	30.00			

3.2 MS Optimization

Due to the different chemistries of the components in smokeless powder, the ionization technique could not detect all compounds exclusively in positive mode or exclusively in negative mode. Negative ionization mode has been found to be the most successful method for analyzing explosives. (44) For explosives to form stable adducts, specific anions are added to the mobile phase. The adducts that are formed by the addition of the anions can only be detected in negative mode. (20) Positive mode is successful in the analysis of stabilizers and other smokeless powder additives. (44) When in positive mode, the working parameters for the MS were as followed: Curtain Gas (CUR) 10.0, Collision Gas (CAD) medium, IonSpray Voltage (IS) 5000.0, Temperature (TEM) 150.0, Ion Source Gas 1 (GS1) 30.0, and Ion Source Gas 2 (GS2) 30.0. When in

negative mode, the working parameters for the MS were as followed: CUR 10.0, CAD medium, IS 3500.00, TEM 150.0, GS1 30.0, and GS2 30.0. Optimization of the IonSpray voltage was completed after the multiple reaction monitoring (MRM) and LC gradient were optimized. Please refer to Table 2 for a summary of the parameters.

Table 2. MS Parameters

	Positive Mode	**Negative Mode**
CUR:	10.0	10.0
CAD:	Medium	Medium
IS:	5000.0	3500.0
TEM:	150.0	150.0
GS1:	30.0	30.0
GS2:	30.0	30.0

Nitroglycerin, ethyl centralite, diphenylamine, and dibutyl phthalate were the four components originally chosen for method optimization. These compounds were targeted because they are characteristic of smokeless powder, and have been detected using similar methods in previous literature. (9) Nitroglycerin, dibutyl phthalate, and diphenylamine standards were obtained from Sigma Aldrich (St. Louis, Missouri). Ethyl Centralite was obtained from Accustandard (New Haven, Connecticut.)

The optimization of each compound was completed by first diluting the standard to the appropriate concentration. The standard operating procedure associated with compound optimization recommended diluting the standard to 100ng/mL. After dilution,

the standard operating procedure was followed specifically for automatic compound optimization on LC/MS/MS using Analyst. To dilute the standards, a diluent appropriate for the optimization was needed. Methanol (Fisher 99.9% purity Optima) with 0.1% formic acid (Sigma Aldrich >95% reagent grade) was used. Diphenylamine, ethyl centralite, and dibutyl phthalate were diluted to a concentration of 100ng/mL and then optimized using positive tuning mode. Diluting nitroglycerin to 100ng/mL proved to be too diluted to detect. Nitroglycerin solutions were made at higher concentrations until optimization was possible. Optimization was possible at 100µg/mL. Nitroglycerin was optimized in negative tuning mode. Table 3 shows the multiple reaction monitoring parameters for the optimization.

Table 3: MRM Parameters

Compound	Ethyl Centralite	Dibutyl Phthalate	Diphenylamine	Nitroglycerin
Ionization Mode	ESI +	ESI +	ESI +	ESI -
Precursor Ion (m/z)	269.20	280.20	170.13	225.81
Product Ion 1 (m/z)	120.04	149.01	92.96	61.88
CXP (Voltage)	8.00	10.00	6.00	-1.00
CE (Voltage)	33.00	19.00	37.00	-16.00
Product Ion 2 (m/z)	77.12	59.00	65.00	162.89
CE (Voltage)	71.00	43.00	45.00	-9.00
CXP (Voltage)	2.00	10.00	2.00	-25.00
DP (Voltage)	71.00	46.00	91.00	-35.00
EP (Voltage)	10.00	10.00	10.00	-10.00

3.3 IonSpray Optimization

After the MRM and LC methods were optimized the IonSpray Voltage needed to be optimized as well. Each compound was run on the same LC gradient. All parameters in the MS were consistent, except for the voltage. After analysis, the abundance of the compounds at each voltage level was compared. For positive mode, the first voltage tested was 2000V. Each test the voltage was increased by 500 volts until capped at 5000 volts. Negative mode also started at 2000V and was ramped up by 500 volts until capping at 4000V. To ensure continuity, the samples were prepared by using smokeless powder from only one round of ammunition. Five grains of smokeless powder were dissolved in 1mL of acetone. A 1:25 sample to the mobile phase A ratio was used for each run. Table 4 summarizes the abundance for each compound at the respective voltage. Figure 8 shows the chromatogram of the optimized voltage in positive mode. Figure 9 shows the chromatogram of the optimized voltage in negative mode.

Table 4. IonSpray Voltage Optimization Study

	Positive Mode (Abundance)		**Negative Mode (Abundance)**
Voltage:	DPA	EC	NG
2000	2.10E+04	2.70E+04	1.60E+04
2500	2.75E+04	2.80E+04	1.80E+04
3000	3.30E+04	3.00E+04	2.10E+04
3500	3.90E+04	3.20E+04	2.30E+04
4000	4.4 E+04	3.30E+04	2.20E+04
4500	5.80E+04	4.20E+04	N/A
5000	6.40E+04	4.50E+04	N/A

With each increase of the IonSpray voltage, the abundance of EC and DPA similarly increased. Although the positive trend between increasing voltage and abundance suggest that an IonSpray voltage higher than 5000 volts could be beneficial to the detection of EC and DPA, 5000 volts is the ceiling of the instrument's capacity. Therefore, with 5000 volts producing the highest abundance for both DPA and EC out of the six tested, 5000 volts was determined to be the optimal parameter for positive mode ionization.

Similar to the positive mode trials, the negative mode trials started at 2000 volts. From 2000 volts to 3500 volts, the relationship between voltage and abundance seemed to mimic the results from the positive mode trials. That trend no longer continued when the voltage was increased from 3500 volts to 4000 volts. Nitroglycerin's abundance dropped slightly with the increase in voltage, and it was therefore determined that 3500 volts would be the optimal parameter for negative mode ionization.

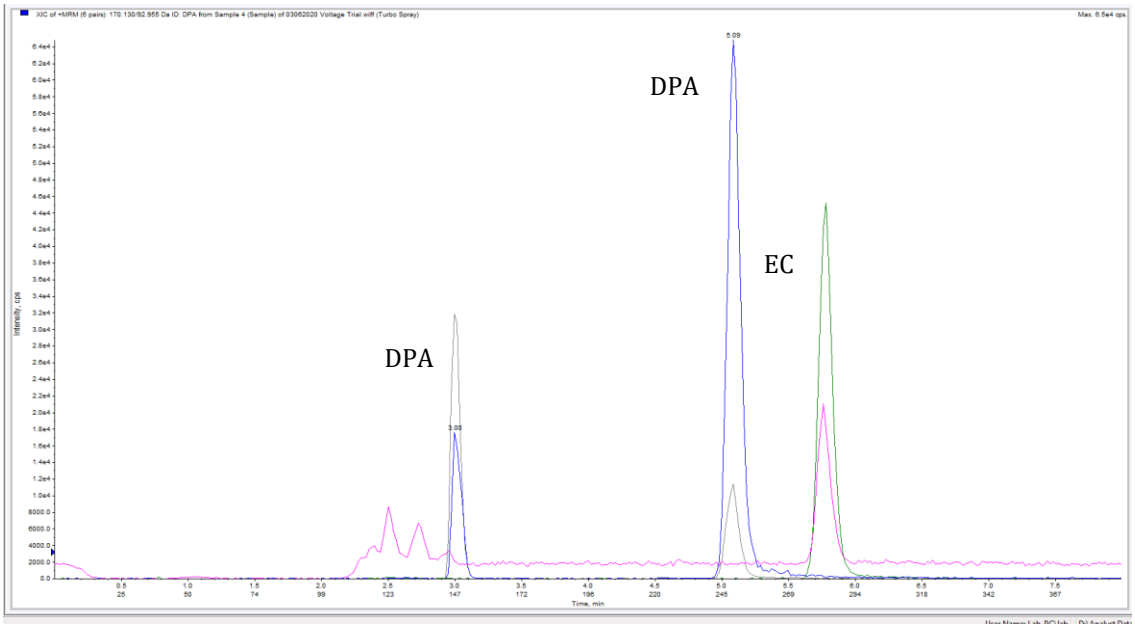

Figure 8. Chromatogram of Voltage 5000 in Positive Mode with DPA and EC Identified

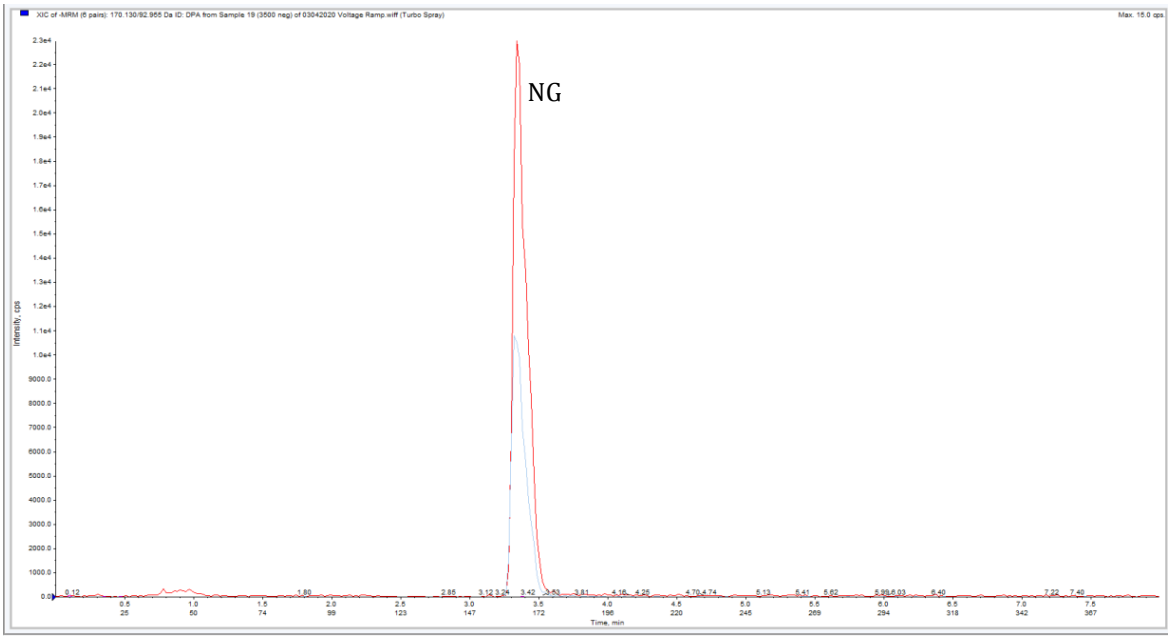

Figure 9. Chromatogram of Voltage 3500 in Negative Mode with NG Identified

4.0 RESULTS AND DISCUSSION

4.1 Removal of DBT from Testing

Dibutyl phthalate is a commonly used plasticizer in smokeless powder. DBT aids in the extrusion portion of production. Aside from smokeless powder, plasticizers can be used and found in various other environmental and occupational occurrences. Due to their high occurrences and sticky nature, plasticizers can often cause contamination or carry-over.

DBT contamination was originally suspected during analysis of a small amount of smokeless powder. There were large amounts of DBT detected and little to none of the other three compounds. The contamination was confirmed in a dilution trial. The purpose of the trial was to determine the limit of detection of the smokeless powder on

the LC/MS. This was also completed to not overwhelm the system with large amounts of smokeless powder, as testing continued. When the trial was concluded, DBT was detected in all samples of dilute smokeless powder as well as all solvent blanks. This showed that there was either carry-over from the samples or there was DBT contamination at some point in the system. To ensure accurate detection of DBT in fired samples, the contamination would have to be located, cleaned out, and prevented.

Locating the possible site of contamination was the first step in ridding the system of DBT. Air samples were analyzed to determine if the samples were causing the reintroduction of the DBT or if the system was internally contaminated. To do this, the method and all solvents were kept the same from the previous runs. Figure 10 shows the chromatogram of the air sample with the DBT peaks highlighted. Both peaks were consistent with DBT, and due to the peak on the right having the same retention time as previous runs, it was concluded that the DBT contamination was internal and not originating from the samples.

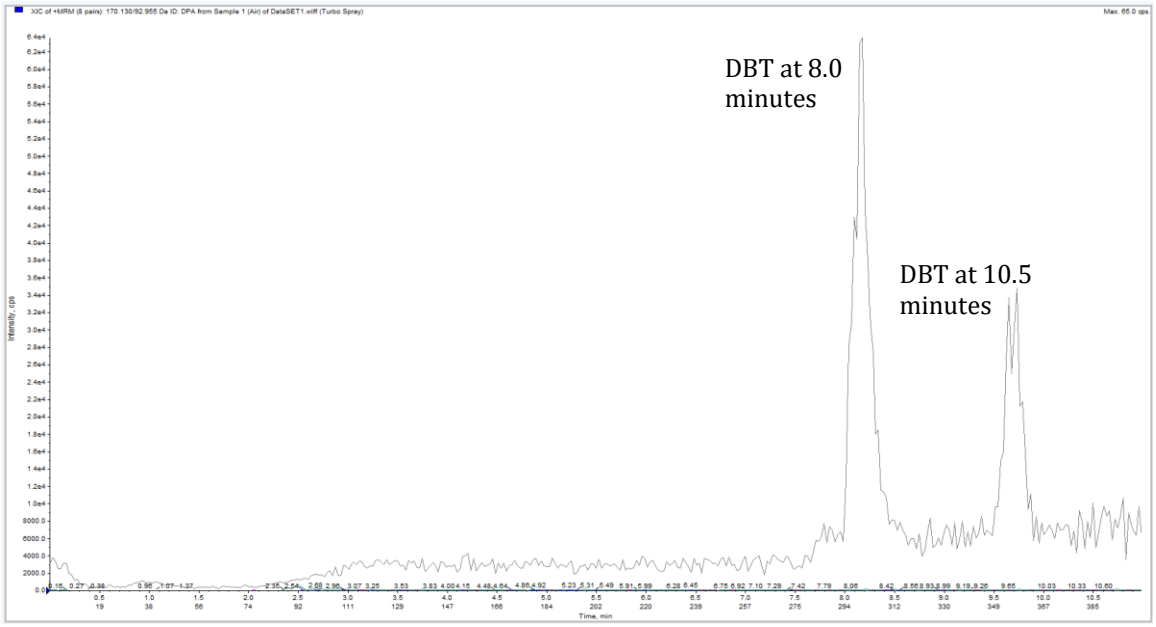

Figure 10. Chromatogram of Air Sample with DBT Highlighted

The next step in locating the contamination included remaking the mobile phases and needle wash solution. The LC/MS used in this experiment was shared with other users and other methods. To eliminate the possibility of another user or method introducing a plasticizer into the solutions, all were remade. Before the solutions were hooked back up to the LC, it was flushed out with King's solution. King's solution is 1-part Cyclohexane, 1-part acetonitrile, and 2-parts isopropanol. The solution replaced both mobile phases and was run through the LC for over an hour. Once the hour was complete, the remade solutions replaced the King's solution and ran through the system for 20 minutes. To check if the solution had effectively cleaned the system, air samples and solvent blanks were analyzed. DBT was detected in the solvent blanks and air samples at approximately 10 minutes. From these results, it was suspected that DBT was being

introduced to the system in another way, therefore the next steps included ridding the method of as many sources of plasticizers as possible.

DBT was eluting at approximately 10 minutes for each sample. Therefore, it was assumed that DBT was being reintroduced during each run. Since flushing out the system and remaking the solution did not eliminate the presence of the contamination, the focus was turned to the mass spectrometer. To ensure the contamination was being contributed by the ion source or mass analyzer, a solvent blank was analyzed though only the MS. When the blank resulted in a lack of the compound of interest, it was confirmed that the contamination was coming from the LC system.

The tubing within the LC and the tubing connecting the LC to the MS is plastic which could have contributed contamination. Resources were not immediately available to change the tubing to non-plastic tubing and other methods of removing plastic were attempted before considering ordering new, non-plastic tubing. LC vials contain a septum that can be made with plasticizers. A solvent blank was prepared in a single glass LC vial without a lid and was run in triplicate. Results from the first run show a high abundance DBT with a retention time of approximately 10.5 minutes. The second and third run detected DBT at 10.5 minutes but the abundance was only around 4000cps. Due to the high abundance of DBT in the first sample, other methods of removing plastic were looked into. Next, all aliquoting was completed with glass pipettes replacing the single-use plastic pipettes used in previous runs. A new solvent blank in a lidless glass vial was tested in triplicate. Similar to the previous results, a high abundance of DBT was detected in the first sample with significantly lesser amounts in the second and third. DBT had a

retention time of 10.5 minutes for all three runs. The final effort to remove DBT from the system was backwashing the LC column. This was completed to loosen and push any contaminants stuck in the column through and then out of system. After the column was backwashed, Mobile Phase A was run through the system which allowed the column to resettle. Finally, a solvent blank was aliquoted with a glass pipette into a vial without a lid. This sample was run in triplicate. The results were consistent with the first two trials. There was a high DBT abundance in the first sample, and less in the final two. An example of the results is shown in Figure 11**.** Due to these strange results and the inability to rid the system of the DBT contamination, the MRM and LC gradient was no longer tailored to detect DBT.

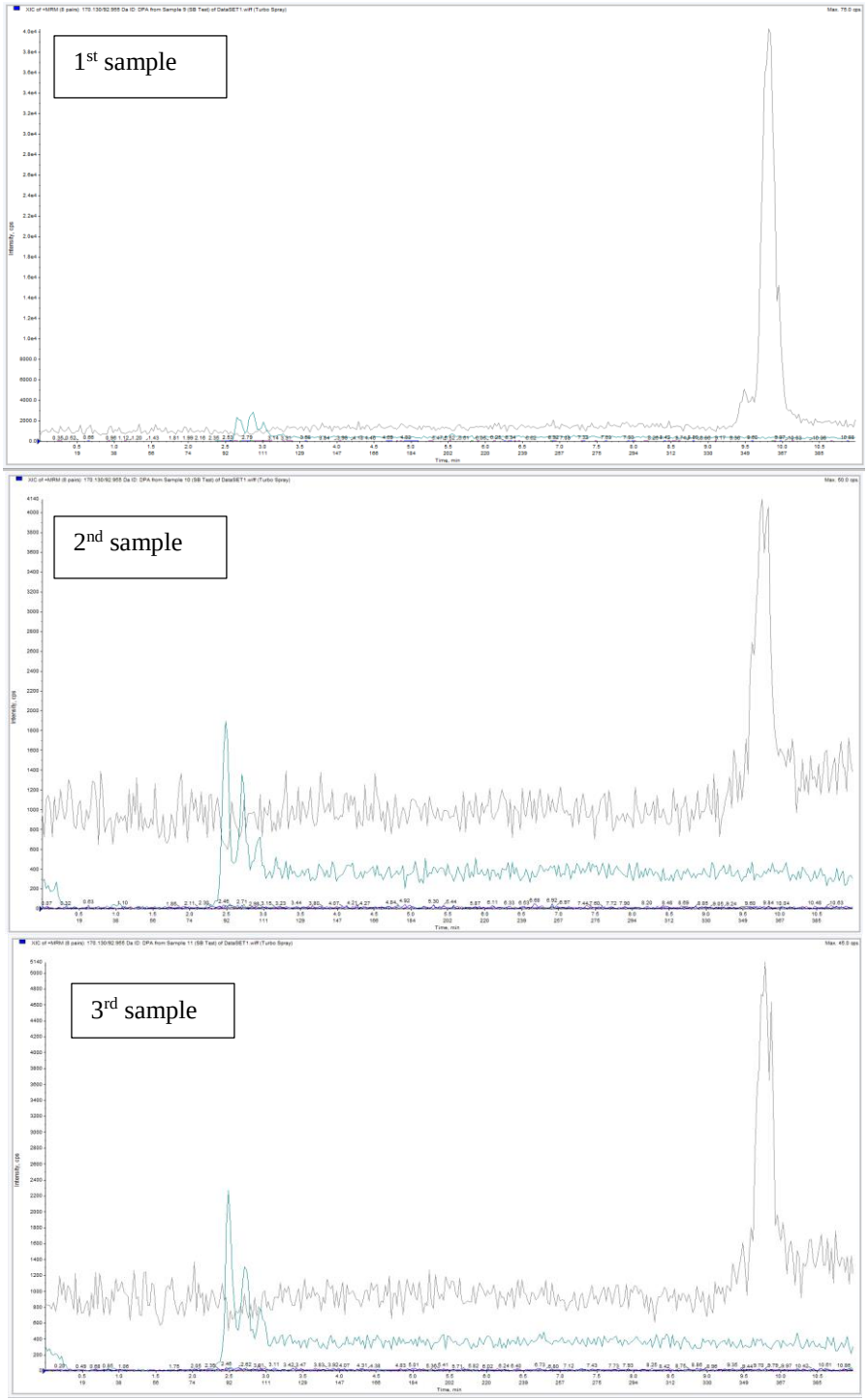

Figure 11. Example of DBT Removal Trial Results

4.2 Future Directions

After the optimization of the LC/MS method, the optimization of both the sampling and elution technique must follow. As mentioned in the literature, there is a wide range of sampling techniques and the elution solvent. This section describes how sampling and elution of OGSR will be accomplished.

A review of the literature does not describe a uniform technique when sampling OGSR from hands. Cotton swabs moistened with isopropyl alcohol is a technique commonly discussed for sampling and the one chosen for this research. Isopropyl alcohol is a common active ingredient in antiseptics and used in the production of cosmetics, chewing gums, and shellacs, and therefore is acceptable to use on human skin. (35) Isopropyl alcohol also effectively recovers organic gunshot residue. Cotton swabs are often used for sampling. They are cheap, effective at picking up residue, and will release the compound of interest with the proper elution solvent. For these reasons, cotton swabs with isopropanol are chosen as the sampling technique.

Once a sampling method was chosen, the next step was to determine where the focus of sampling would take place. Refer to Figure 12 which shows the proper way to hold a firearm with the dominant hand. The shooter in the picture is holding the firearm with their left hand. Notice that the thumb, index finger, and webbed area between the two are in closest proximity to the slide of the firearm. The slide will allow the firearm to reload, but that action also allows a significant amount of the gases to be released. Due to this, and the former research completed by Ali et al, the aforementioned areas will be the focus of sampling. (14)

Figure 12. Shooter Holding a Firearm with their Dominant Hand

The same method of sampling will be used for each sample and is as follows. First, the cotton swab pack will be opened, and the swab will be fully dipped in a 4mL vial containing 2mL of isopropyl alcohol. The excess solution will be pushed off the swab by pressing it up against the sides of the vial. Excess solution when sampling could wash away potential residue or dilute the compounds of interest. With continuous rotation, the moistened cotton swab will be rubbed along the back of the hand and wrist for 30 seconds. 15 seconds are dedicated to the high focus area (green), 10 seconds to the middle and ring fingers (yellow), and 5 seconds dedicated to the rest of the hand (red). This technique will be repeated, with the same swab, on the palm of the hand. Following the completion of the first sampling run, the high focus areas on the back of the hand will be sampled again for 15 seconds followed by the high focus areas on the palm for 15 seconds. With sampling completed, the cotton swab is placed into a 4mL vial with

approximately 1mL of elution solvent. If the cotton swab has a stick that prevents the lid from being tightened, the stick should be snapped so the vial can be sealed. Figure 13 shows the areas of focus and sampling times.

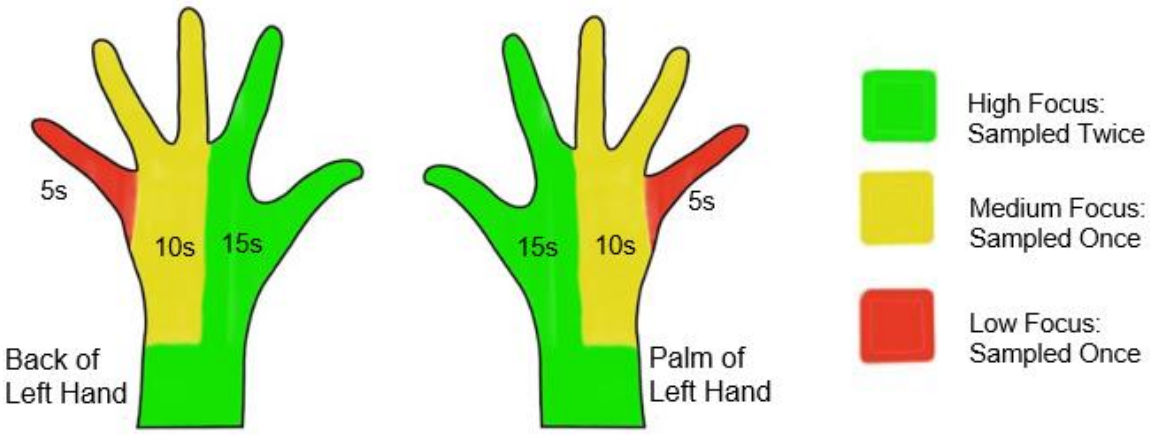

Figure 13: Map of Sampling on Left Hand

Similar to information about the sampling technique, there was conflicting information about which elution solvent would provide the best results. From the literature, three solvents were often mentioned and chosen to participate in a study. These solvents were acetonitrile, methanol, and a 50/50 mixture of these two solvents. To determine which solvent would be the most effective with the optimized LC/MS method, a solvent study was devised.

Using the sampling technique described above, the proposed elution study involves sampling OGSR from shooters hands. Sampling would take place immediately after firing, and would be completed with the three proposed solvents. After sampling, the cotton swab with the residue sample would be placed into a 4mL glass vial that

contained approximately 1mL of the respective elution. Vials would be placed in the sonicator's water bath and sonicated for 15 minutes at the lowest temperature the machine allows. Running samples at the lowest temperature, which was 68F degrees for this instrument, is imperative due to nitroglycerin degrading at low temperatures. After sonication, the cotton swab would be removed from the vial with attempts to retain as much elution solvent as possible. This was done by pushing the swab up against the inner walls of the vial to push out any of the liquid. From there, the solution was removed from the vial and poured into a test tube that was conducive to a nitrogen evaporator. Samples would be dried down with room temperature nitrogen and reconstituted with approximately 200µL of mobile phase A. The reconstituted samples would be analyzed using the LC/MS and the abundance of each compound would be compared to the elution solvent. An example of how the abundances would be easily compared is shown below in Table 5. Results from this would give a clear choice to which solvent would work best for this experiment.

Table 5: Proposal of Elution Solvent Study

Elution Solvents vs. Abundance from Shooters Hands				
	Elution Solvent:	**Acetonitrile**	**Methanol**	**50/50 Acetonitrile/Methanol**
Compound:				
DPA				
EC				
NG				

4.2.1 Time course study

Once all parts of the experimental method are optimized, the focus is turned to the time course study. The goal of this experiment is to determine how long OGSR can be detected off of a shooter's hands while they went about their daily lives. To accomplish this a time-course study will be implemented.

The length of the time course will not have a definite end time point. The time points will continue to extend as long as the compounds of interest are being detected. Once the compounds of interest are no longer detected, the time course would cease. The ideal length is 8 hours, where each hour is a time point. Prior to firing, the shooter will wash their hands with soap and dry them with paper towels. Blanks will be sampled immediately after drying their hands. The shooter will then load the firearm with three rounds of ammunition, and discharge all three rounds into a snail trap. The purpose of the

snail trap is to catch and slow the projectile so it does not cause any damage. Sampling of the hands will occur at the appropriate time point after shots were fired.

Time zero, or sampling immediately after firing, will be the baseline for the detection of all other compounds. Time zero will be taken at the beginning of the day before any potential interference from work can contaminate the shooter's hands. Each time point will take place on a new day so that any previous shooting will not obscure the data. Outside time point zero, the shooter will fill out a survey throughout the day. The survey, shown below in Figure 14**,** contains two parts. Part 1, which is filled out throughout the day, considers how many times the shooter washes their hands with soap and water, dries their hands with heated air driers, dries their hands with towels, and has a section to document other non-handwashing liquids that come in contact with the shooter's hands. If an earlier time point does not detect residue, experimenters could turn to the survey to check if daily activity could have been the cause. Part 2, which briefly documents the condition of the hands when sampling, would be completed by the sampler. Part 2 of the shooter survey documents the elution solvent used, the condition of the hands, and if there were any compounds of interest in the blank hand sample.

Date: _____ Shooter: _____

Collected by: _____ Time Point (hours): _____

Section 1: To be completed by *Shooter*

Since time of shooting please document the time that each event occurred:

Event:	Time(s) Event Occurred:	Tally
Washed Hands with Soap		
Dried Hands w/ Paper or Cloth Towel		
Dried Hands with Air Dryer		
Used alcohol-based hand sanitizer:		
Put lotion or other moisturizer on hands:		
Miscellaneous **fluids** on hands (i.e. blood, grease, solvents, etc.)		
Any other necessary reports.		

Section 2: To be Completed by *Sampler*

Condition of Hands	
Collection Solvent	
Elution Solvent	
Compounds of Interest on Blank	

_____	_____	_____
Signature Shooter	Name Shooter (please print)	Date
_____	_____	_____
Signature Sampler	Name Sampler (please print)	Date

Figure 14: Shooter Survey

4.2.2 Next Steps

Given that the time course study provided conclusive results, this experiment could be taken further in a myriad of directions. Some of these directions include optimizing a method that could also quantify the compounds, adding additional compounds of interest, using different ammunitions, and various styles of firearms.

Optimizing the method to quantify the compounds of interest would allow for even further experimentation. Using the same smokeless powder composition, residues from different styles of firearms could be analyzed to determine if a certain style released more residue than others. Different compositions of smokeless powder could be used with the same firearm to determine if a different style of powder or ammunition causes various amounts of residue detection. Quantification could lead to the ability to calculate whether more shots fired correlates to the increase in detected residue.

The three compounds detected in this study are only a select few of the extensive compounds of interest in smokeless powder. Adding more compounds to the method would allow results to carry more weight when used as evidence. As stated earlier there are no known environmental or occupational occurrences of the combination of all smokeless powder components. Therefore, adding more components to the current method solidifies and adds weight behind declaring unknown samples as OGSR.

Different styles of ammunition, including different brands, different morphology of grain, or different smokeless powder additives, could also test this method's depth. Testing different styles would question whether the current method has the ability to detect the compounds of interest from a different ammunition source. If the current

method does not have the depth to successfully test different ammunition styles, what steps could be taken so that a new method would not have to be produced for each style. Finally, as new ammunitions are tested, does this method have the capability to become a universal method for testing OGSR? If not, how does it get there.

5.0 CONCLUSION

Inorganic gunshot residue has been in the spotlight since the beginning of GSR testing. Starting as a simple color test, it has grown into a well-documented analysis on a Scanning Electron Microscope. As ammunition advances, more primers are being made without the addition of lead or other heavy metals. Even before the switch, the disadvantages of IGSR include its transient nature and the higher likelihood of encountering particles in environmental or occupational occurrences. Because of this, a shift in the analysis is required.

Organic gunshot residue has been researched to add more weight to IGSR. Unlike IGSR, OGSR does not have a transient nature that IGSR has proven to have. There is also a smaller possibility of OGSR being detected due to an environmental or occupational occurrence. OGSR analysis started at TLC and has graduated to detection with HPLC/MS/MS. HPLC provides low-temperature separation aided by a diversity of mobile phases and columns. Detection with MS allows for customizable ionization techniques thereby leading to successful analysis of all compounds in OGSR.

A successful LC/MS method requires the optimization of the sampling technique, the elution method, and the instrumental method. In this experiment, the LC/MS method was optimized using smokeless powder from ammunition and reference standards. The next steps for the experiments were designed to include an elution solvent study and a time-course study.

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