DEVELOPMENT OF CHROMATOGRAPHY AND
MASS SPECTROMETRY METHODS FOR EXPLOSIVES ANALYSIS

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Novel methods for the analysis of explosives and related compounds were developed in this research. A gradient reversed-phase high-performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESIMS) method was used in the positive ion mode for the analysis of the organic additives in smokeless powders. Gas chromatography (GC) methods with electron capture detection (ECD) and negative chemical ionization mass spectrometry (MS) were developed for the separation and detection of common high explosives. Negative ion ESIMS was used for the detection of high explosive adducts. An isocratic HPLC-ESIMS method was evaluated for the simultaneous analysis of high explosives using the negative ion mode.

Smokeless powders are used as propellants for small arms ammunition but have also been used as the energetic material in improvised explosive devices such as pipe bombs. Smokeless powders contain organic additives that facilitate production procedures, prevent degradation, and enhance energetic efficiency. The gradient HPLC-ESIMS method was used to determine compositional differences among smokeless powders by simultaneously quantifying the organic additives. The relative ESIMS intensities of the powder constituents were also evaluated as a function of the solution parameters associated with the gradient elution method. The newly developed HPLC-
ESIMS method was used for the analysis of different unburned smokeless powders and pipe bomb samples containing these powders.

Two GC methods were developed for the separation and detection of high explosives using selective detectors. The application of GC/ECD and GC with negative chemical ionization mass spectrometry allowed for the quantitation of nitrated explosives. Detection limits in the ng/mL range were determined using the GC/ECD method. The GC-MS instrument was equipped with a large bore capillary column that was split to a fused silica transfer line to minimize the thermal degradation of the energetic compounds.

The negative ion ESIMS detection of adducts of high explosives with chloride, formate, acetate, and nitrate was accomplished. The relative stability of the adduct species was determined to illustrate the gas-phase interaction of neutral explosives with the anions. An isocratic HPLC-ESIMS method was developed for the separation and detection of selected high explosives as anionic adducts to provide structural information that can be used for compound identity.

Approved

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List of Abbreviations

ACN ...............Acetonitrile
AN ..................Ammonium Nitrate
ASTM .............American Society for Testing and Materials
ANOVA ..........Analysis of Variance
AOAC ............Association of Official Analytical Chemists
APCI...............Atmospheric Pressure Chemical Ionization
CRM...............Charged Residue Model
$r^2$ ...............Coefficient of Determination
CI....................Chemical Ionization
CID.................Collision Induced Dissociation
RDX ...............Cyclo-1,3,5-Trimethylene-2,4,6-Trinitramine
HMX ............Cyclo-1,3,5,7-Tetramethylene-2,4,6,8-Tetranitramine
DBP ...............Dibutylphthalate
DEP ...............Diethylphthalate
DMP ...............Dimethylphthalate
24DNDPA......2-4′-dinitrodiphenylamine
44DNDPA......4-4′-dinitrodiphenylamine
26DNT ..........2,6-Dinitrotoluene
DPA..............Diphenylamine
DLI..............Direct Liquid Introduction
ECD.............Electron Capture Detector
EI.................Electron Impact Ionization
ESIMS............Electrospray Ionization Mass Spectrometry
EGDN.............Ethylene Glycol Dinitrate
EC................Ethyl Centralite
EIC ..............Extracted Ion Chromatogram
FBI ..............Federal Bureau of Investigation
GC .............Gas Chromatography
DB5 ............GC Column Stationary Phase (95% dimethyl 5% diphenyl polysiloxane)
$\Delta G_f$.........Gibbs Free Energy of Formation
GSR.............Gun Shot Residue
HPLC ..........High-Performance Liquid Chromatography
IPA .............Isopropanol
IET ............Ion Evaporation Theory
kJ..............Kilojoule
kV...............Kilovolt
MS..............Mass Spectrometry
$m/z$ ...........Mass-to-Charge Ratio
MeOH..........Methanol
MC .............Methyl Centralite
min. .............Minute
mM.............Millimolar
NCI ............... Negative Chemical Ionization
NC ................ Nitrocellulose
4sDPA .......... 4-Nitrosodiphenylamine
NsDPA .......... N-Nitrosodiphenylamine
2NDPA......... 2-nitrodiphenylamine
4NDPA......... 4-nitrodiphenylamine
NG ............. Nitroglycerin
4NT .......... 4-Nitrotoluene
Tetryl......... 2,4,6,N-tetranitro-N-methylaniline
C_{18} .......... Octadecylsilane
ODS .......... Octadecylsilane
C_{8} .......... Octylsilane
ppm .......... Parts per million
ppb .......... Parts per billion
PETN .......... Pentaerythritol Tetranitrate
psi .......... Pounds per square inch
QITMS ......... Quadrupole Ion-Trap Mass Spectrometer
r ............... Radius, Distance
RSD .......... Relative Standard Deviation
k' .......... Retention Factor, Capacity Factor
{t_r} .......... Retention Time of Solute
{t_0} .......... Retention Time of Unretained Species
SSI.................Shimadzu Scientific Instruments
SPE................Solid Phase Extraction
SPME..............Solid Phase Microextraction
s.d. ...............Standard Deviation
TEA...............Thermal Energy Analyzer
TNT...............2,4,6-Trinitrotoluene
EPA...............United States Environmental Protection Agency
I. Chapter 1. Introduction

Explosives are designed to perform work and are generally recognized as energetic materials. While explosives have many legitimate uses like mining and construction, they have also been used in criminal acts by terrorists. As a result of the illegal use of explosives, investigators are faced with providing critical information that often makes use of instrumental techniques to detect and identify energetic compounds. The procedures used for the analysis of explosives should provide conclusive results for a particular sample in a timely manner. Extensive research has gone into improving instrumental analysis methods for explosives analysis but recent developments have focused on a narrow range of samples for a limited number of energetic compounds. Because each forensic sample is unique, the newly developed methods are not capable of detecting a variety of explosives. The development of comprehensive analysis methods are needed to provide efficient procedures that can be applied to explosives samples by forensic laboratories.

A. Overview of Explosives

Explosives have been grouped by various measures such as application and chemical structure. The common elemental constituents in organic explosives include carbon, hydrogen, nitrogen, and oxygen.¹ The chemical reactions that occur during an explosive event result in the breakdown of energetic materials into more stable compounds. For example, a molecular explosive containing the elements listed above react to form water and carbon dioxide as well as hydrogen, oxygen, and nitrogen gases.
In the forensic analysis of explosive residue, categorizing energetic materials provides particular descriptions that influence the laboratory procedures used for identification. Explosives are categorized in two distinct classes with respect to the decomposition rate of the energetic components. The difference between the two classes of explosives is in the propagation of the explosion event from the reacting materials to unreacted explosive. High explosives undergo exothermic decomposition as a result of an initial shock wave by breaking chemical bonds at a characteristic velocity through the unreacted material. The decomposition of high explosives is known as detonation and the shock wave event continues in all directions, which creates high pressures and temperatures. Examples of high explosives include dynamite, C-4, and Semtex.

Low explosives react slower and deflagrate from the initiation point through the energetic material away from the unreacted material. Deflagration is a form of rapid combustion that is subsonic in propagation. The distinction should be made between detonation, deflagration, and incidents as a result of vapor explosions, which is caused by combustion. As a result of the reactions involved in deflagration, high temperatures are produced but confinement is required to create high pressures. When low explosives are confined, large quantities of gas are produced and the resulting pressure causes the container to rupture. Examples of low explosives include black powder, pyrotechnics, and smokeless powders. Because deflagration proceeds away from the reaction front, low explosives are consumed by the decomposition of one particle to the next. The principle of transferring energy through the material in a linear manner makes low explosives ideal for use as propellants.
Propellants are low explosives, which contain a mixture of ingredients that serve as fuel, oxidizer, and sensitizer when combined in specific proportions. The most commonly used propellant in small arms ammunition is smokeless powder. Smokeless powders are composed of nitrocellulose and other organic additives. The additive compounds are used to facilitate processing, enhance energetic efficiency, and prevent degradation during storage. Other applications also utilize propellants, such as pyrotechnics, air bag initiators, and hobby rockets. Because propellants are commercially available for muzzle loading and reloading ammunition, a potential problem for forensic investigators is the use of these propellants in improvised explosive devices such as pipe bombs.

Although these energetic materials are considered low explosives, the power of propellants should not be underestimated as they are extremely dangerous when confined. While the deflagration of low explosives occurs at lower temperatures and pressures than the detonation of high explosives, these materials can cause severe damage when confined in a small container. Because the decomposition of low explosives proceeds away from the unreacted material, a portion of unburned material may be recovered from the crime scene. Forensic investigators utilize these intact materials, in addition to residue to identify the propellant.

1. Smokeless Powders

Smokeless powders are mainly composed of nitrocellulose (NC). These propellants are prepared mechanically by extrusion. Smokeless powders are arranged
into three classes based on the energetic material added during the manufacturing process. The distinction among the different classes of smokeless powders based on their application can be described by the added energetic material. Single-base powders consist of only NC and are used exclusively in small arms ammunition such as rifles cartridges. Double-base smokeless powders contain nitroglycerine (NG) as well as NC. By using the additional energy provided by NG, double-based powders are used in ammunition for pistols and shotguns. Triple-base propellants, which are predominately used in large caliber ammunition for military purposes, incorporate nitroguanidine in addition to NC and NG.

During the manufacture of smokeless powders, various compounds are incorporated, which are used as modifiers and stabilizers. Chemical modifiers such as dibutylphthalate (DBP) are used as plasticizers, which aid in maintaining powder shape during production. Stabilizers, such as diphenylamine (DPA) are added to suspend the decomposition of NC. The degradation products of NC and water include nitrous and nitric acids, which are scavenged by DPA. The resulting reaction products include nitroso and nitro derivatives of DPA, such as N-nitrosodiphenylamine (NsDPA) and 4-nitrodiphenylamine (4NDPA). Other organic compounds are frequently added to smokeless powders, which serve a dual purpose. For example, the \( N,N' \)-dialkyl-\( N,N' \)-diphenyl urea based compounds called centralites are considered to be plasticizers and stabilizers, which aid in manufacturing as well as extend the shelf life of the propellants. Nitrotoluene and dinitrotoluene isomers, such as 4-nitrotoluene (4NT) and 2,6-
dinitrotoluene (26DNT) are used as plasticizers to facilitate processing but may undergo the nitration reactions like the stabilizers in smokeless powder.

Each powder contains a mixture of components, some commonly used more than others. The different compounds in smokeless powders may represent a unique chemical profile. However, the composition of smokeless powders is manufactured to maximize power generation, which may not result in a particular profile. In addition, reworking and recycling of powders often occurs in the process of production and distribution. These procedures may result in different powders being mixed which have their own individual chemical profiles. For example, if powders within a batch are found to be unsatisfactory, they may be reworked into the production process for use in another lot. Recycling occurs when unused smokeless powders are returned to the producers and make their way into a different powder lot.
Figure 1.1. Structures of smokeless powder additives. a. Dialkylphthalate plasticizers: dibutylphthalate (DBP), diethylphthalate (DEP), diethylphthalate (DMP). b. Diphenylamine (DPA) stabilizers and nitro derivatives: $N$-nitrosodiphenylamine (NsDPA), 4-nitrosodiphenylamine (4sDPA), 2-nitrodiphenylamine (2NDPA), 4-nitrodiphenylamine (4NDPA), 2-4'-dinitrodiphenylamine (24DNDPA), and 4-4'-dinitrodiphenylamine (44DNDPA). c. $N,N'$-dialkyl-$N,N'$-diphenyl urea based centralites: methyl centralite (MC), and ethyl centralite (EC).
2. High Explosives

Molecular high explosives contain covalently bonded carbon, nitrogen, oxygen, and hydrogen. The main feature of organic high explosives is the nitro group (-NO₂). These elemental constituents function as the energetic components within the same molecule. The organic components of the high explosive serve as fuel, oxygen as the oxidizer, and the high energy bonding of the nitro group as the sensitizer. The most common organic high explosives are divided into three classes based on the chemical bonding to -NO₂. The three classes include nitrate esters, nitramines, and nitroaromatics as shown in Figure 1.1. Nitroglycerine (NG), ethylene glycol (EGDN), pentaerythritol tetranitrate (PETN) are nitrate esters that are distinguished by the R-O-NO₂ bond. Nitramines such as cyclo-1,3,5-trimethylene-2,4,6-trinitramine (RDX), and cyclo-1,3,5,7-tetramethylene-2,4,6,8-tetranitramine (HMX) contain an R-N-NO₂ bonding sequence. The nitroaromatic explosives are characterized by the Ar-NO₂ bond and examples are trinitrotoluene (TNT) 2,6-dinitrotoluene (26DNT), and 2,4,6,N-tetranitro-N-methylaniline (tetryl).

The differences in the chemical structures as well as other physical properties influence the applications for which the organic molecular explosives are used. Although nitrated organic explosives can be used alone, more recently, mixtures have been developed. While NG was the original explosive used in dynamite, EGDN has been added to reduce the freezing point for low temperature conditions. The physical properties are also important considerations for explosives analysis, which range from
sample collection to instrumental analysis. For instance, the relative vapor pressures of explosives have important implications for the trace detection of explosives. The volatility of the three classes of explosives varies widely both among the common explosives and within each class. For example, NG and EGDN are highly volatile but their vapor pressures are several magnitudes higher than that of PETN.1

B. Forensic Application of Explosives Analysis

Because trace levels of explosives remain as residue following a bombing incident, a sensitive method of detection is necessary to determine the presence and identification of explosives in forensic samples. The detection and identification of energetic materials depend on the results of initial “presumptive” tests with additional independent confirmatory detection by instrumental methods. While presumptive or screening tests range from simple spot tests to instrumental analysis, many provide comparative results that must be confirmed. For example, certain screening tests involve the separation of explosives using gas chromatography (GC). When used with universal detectors, GC methods only provide relative retention time data. Traditionally, the detection of explosives residue has been performed by GC with mass spectrometry (MS) as a confirmatory method.6,7 Because MS is capable of providing structural information, the detection of explosives confirms the results of presumptive or screening tests.
Figure 1.2. Structures of common high explosives. a. nitrate esters: ethylene glycol dinitrate (EGDN), nitroglycerin (NG), and pentaerythritol tetranitrate (PETN). b. nitramines: cyclo-1,3,5-trimethylene-2,4,6-trinitramine (RDX), and cyclo-1,3,5,7-tetramethylene-2,4,6,8-tetranitramine (HMX). c. nitroaromatics: trinitrotoluene (TNT), picric acid, 2,6-dinitrotoluene (26DNT), and 2,4,6,N-tetranitro-N-methylaniline (tetryl).
Due to the different physical properties of explosives, limitations of GC methods have been established for particular compounds. For example, the GC-MS analysis of thermally labile explosives at trace levels gives rise to results that are insensitive or indistinguishable due to thermal instability in the GC injector or MS ionization source. In response to the problems associated with GC-MS, alternate analysis methods for explosives detection have been developed. High-performance liquid chromatography (HPLC) addresses particular shortcomings of GC methods because the separation is carried out at room temperature. Because mass spectrometry provides valuable information for sample identification, several HPLC-MS methods have been developed for the analysis of explosives. Atmospheric pressure ionization techniques such as electrospray ionization mass spectrometry (ESIMS) have been coupled with reversed-phase HPLC methods for the detection of specific explosives. However, comprehensive HPLC-ESIMS analysis methods for explosives and related compounds have not been demonstrated.

While the drawbacks of GC methods have limited the use of this technique to volatile explosives, there is a need for robust methods for the forensic analysis of trace organic explosives. Recent developments in GC applications have allowed for the detection of specific compounds that were previously difficult to detect. While these developments have been illustrated for a limited number of energetic materials, slight modifications to these methods allows for the detection of the most common high explosives compounds encountered during forensic investigations. In addition, there is a demand for the development of HPLC-MS methods capable of providing quantitative and
definitive structural information for the forensic chemical analysis of explosives. HPLC-ESIMS is an effective technique for the detection of thermally labile compounds. However, the implementation of this technique in forensic laboratories has been limited to detecting specific compounds because comprehensive methods for the analysis of smokeless powders and high explosives have not been demonstrated.

C. Aims of This Research

The goal of this research is to develop and validate high-performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESIMS) and GC methods for the detection of explosives and related compounds. A gradient HPLC-ESIMS method was used for the analysis of smokeless powder additives. By identifying and quantifying the particular additives in different powders, the HPLC-ESIMS method allowed for the determination of minor variations in the levels of the organic powder constituents. The results provide quantitative information to differentiate several powders by their chemical profile. The solution parameters associated with the gradient elution method were characterized to evaluate the ESIMS ionization efficiency of selected smokeless powder additives in the positive ion mode.

GC methods with electron capture detection (ECD) and negative chemical ionization (NCI) mass spectrometry were developed for the analysis of common high explosives using wide bore capillary columns (0.53 mm diameter) with high flow rates to minimize the thermal degradation of the energetic compounds. These novel methods will be used for the joint project with the Federal Bureau of Investigation (FBI) in future
experiments to determine the background levels of trace organic explosives in public places. The design of the project involves using GC/ECD for sample screening and GC-MS with NCI for sample identification and confirmation. The results will provide baseline levels of explosives in public places, which will allow investigators to determine the significance of finding explosives following a bombing incident. The portion of the project included in this research involved methods development for the separation and detection of six high explosives using the two GC systems with selective detectors.

In addition, an isocratic HPLC-ESIMS method was developed to identify energetic materials by detecting negative ion adducts of the high explosives with chloride, formate, acetate, and nitrate. By detecting adduct species for each explosive, this approach provided specific structural information that can be used to identify explosives. The characterization of the explosives-adduct species was used to determine the relative stability of the negative ion adducts. The novel HPLC-ESIMS method was applied to the simultaneous analysis of common high explosives.

In each project, method validation was performed with the intention that these techniques will be amendable for use in forensic laboratories. The practical application of the methods were considered with regard to developing more comprehensive analysis techniques. The methods were developed to improve the capability of investigators in the examination of explosives samples.
II. Chapter 2. Instrumental Analysis of Explosives: Theory and Applications

A. Introduction

The forensic analysis of explosives takes advantage of several factors that are used to determine the most appropriate method of analysis. Differences in the physical properties, such as the volatility of organic explosives, gives rise to specific descriptions that influence the analysis procedures used for sample identification. Depending upon the amount of material available and the complexity of the matrix, sample preparation is performed prior to an instrumental analysis method.

Chromatography is the most common instrumental methods used for explosives separation. However, the identification of explosives is difficult with a separation technique alone. Chromatographic identification of explosives requires the use of two separate separation modes, such as two GC analyses with distinctive columns that contain distinctly different stationary phases. Because retention time data provided by chromatographic methods cannot rule out the presence of other components, a more specific identification method must also be used. For example, the analysis of nitrated high explosives by GC makes use of selective detectors such as electron capture detector (ECD) and negative chemical ionization (NCI) mass spectrometry. These detection methods provide sensitive results due to the inherent electronegativity of the nitro group and are feasible owing to the high volatility of certain nitrated high explosives.

Chromatography coupled with mass spectrometry is the most informative method for the identification of the energetic material in complex samples. GC has traditionally been used for the separation of explosives and MS has been the detection method of
choice for explosives identification. HPLC has been used for the separation of explosives to overcome the limitations of GC methods. HPLC has also been employed with universal and selective detectors. Because MS is the preferred detector for sample identification, HPLC-MS has been used for the analysis of specific explosives.

B. Sample preparation

Sample preparation is necessary for the analysis of explosives because most analytical instruments cannot handle sample matrices directly. Because forensic laboratories rarely encounter the exact same type of sample in the same matrix, the procedures used for the analysis of explosives are not always strict protocols. The procedures for the analysis of explosives include several steps: sampling, extraction, and instrumental analysis. In the forensic examination of high explosive residue, established sampling methods are contingent on the type of sample under question. For example, cotton swabs are often used for sampling materials such as large pieces of metal debris.

The most common sample preparation technique is liquid extraction. Samples such as soil may be extracted directly by aqueous or organic solvents. When samples cannot be extracted directly, a sampling procedure is used such as cotton swabs. The explosives are typically extracted from cotton swabs or other material by organic solvents. Additional sample preparation steps can also be used prior to instrumental analysis. The liquid extraction of organic explosives may include a subsequent preconcentration step. For example, soil may be extracted with an organic solvent followed by volume reduction by evaporating the solvent. Other preconcentration steps
such as solid phase extraction (SPE) or solid phase microextraction (SPME) have been used for the analysis of explosives using a variety of instrumental techniques.\textsuperscript{1,10,14-18}

The most commonly used sample preparation techniques are simple but may be limited with respect to specific compounds. While many sample preparation applications are sample or compound specific, their use as a comprehensive sample preparation technique is limited. Due to the wide range of polarities among high explosives and smokeless powder additives, a variety of organic solvents have been used for liquid extraction. For example, previous studies have demonstrated that acetone is effective for the extraction of high explosives from cotton swabs.\textsuperscript{8,10,19} The extraction of smokeless powder additives has been performed using methylene chloride from unburned powders.\textsuperscript{20-23} Because smokeless powders are mainly composed of nitrocellulose, certain organic solvents may cause this polymer to dissolve or swell.\textsuperscript{23} For example, polar organic solvents such as methanol and acetone dissolve the nitrocellulose, which must be removed prior to instrumental analysis. Methylene chloride does not result in the dissolution of nitrocellulose but instead causes the polymer to swell. By using methylene chloride for the extraction of smokeless powder additives, efficient recovery of the constituents is achieved and instrumental analysis can be performed immediately.

C. Gas Chromatography

The separation of nitrated explosives has traditionally been performed by GC.\textsuperscript{7,8} Because certain energetic materials are nonvolatile and thermally labile, the separation of explosives by GC is not easily accomplished. The temperatures required in GC methods
cause some high explosive compounds to decompose. For example, the nitramine HMX and nitrate ester PETN are considered nonvolatile and “frangible.”\(^7\) These compounds easily break down when energy is applied, such as in GC injection ports. To overcome these drawbacks, specialized sample introduction techniques have been developed.\(^8\) The injection temperatures used for the analysis of explosives is typically lower than conventional GC methods. In addition, on-column and direct flash injection techniques have been used to reduce the exposure of the analytes to the high temperatures and activated regions in the injection port. Activated portions in the injector arise from the deposition of reactive compounds from the sample matrix or decomposition products. However, these specialized techniques are not widely available in forensic laboratories.

The GC analysis of explosives has been used with universal detectors such as flame ionization detection and electron impact ionization mass spectrometry. Due to the specificity of nitrated explosive compounds and to increase the sensitivity with complex samples, selective detectors have been used. The most common selective GC detectors include electron capture detector (ECD) and thermal energy analyzer (TEA). The TEA is a chemiluminescence based detector that is selective to compounds containing NO and NO\(_2\).\(^{1,8}\) For the analysis of explosive by GC-MS, NCI is selective to energetic compounds. The NCI mode takes advantage of the electronegativity of nitrated explosives by producing negative ions via resonance electron capture of thermal electrons.
1. Electron Capture Detection

The ECD is a selective gas phase detector for compounds containing electronegative groups such as halogenated or nitrated organics. The ECD operates by supplying electrons from a radioactive source to a biased collection electrode, which is located in the path of the GC effluent stream as shown in Figure 2.1. The ECD signal is derived from a decrease in the current produced by the capture of electrons by compounds in the GC effluent. Because the sensitivity of the ECD for hydrocarbons is poor, the selectivity for electronegative substituents makes this a suitable detector for explosives analysis. One particular shortcoming of this method is that cleanup procedures are often required for “dirty” post-blast samples for the trace detection of explosives.\(^1\)

The use of GC/ECD for the analysis of explosives samples have recently focused on demonstrating specialized sample preparation techniques. The thermal desorption of explosives into a modified GC/ECD instrument was shown for the trace analysis of explosives residue from dry surface wipes.\(^2\)\(^4\) GC/ECD was also used for the analysis of explosives following applications using SPME with different samples. The optimization of SPME conditions for the preconcentration of explosives in aqueous extracts was established using GC/ECD.\(^1\)\(^4\) SPME was also used for the preparation of soil samples containing nitramine explosives followed by GC/ECD analysis.\(^2\)\(^5\) While these studies illustrate the application of novel sample preparation techniques using GC/ECD, the results were verified by GC-MS.
Figure 2.1. Schematic of the electron capture detector. Adapted from 6890 Series Gas Chromatograph Service Manual.\textsuperscript{26}
2. Mass Spectrometry

Following GC, MS has been the most common detection method. MS detection has been recommended for the forensic analysis of explosives due to its ability to provide compositional information. The GC-MS analysis of certain explosives and related compounds using universal ionization such as electron impact can lead to indistinguishable or insensitive results due to thermal instability. The high temperatures required for sample introduction in the GC injector as well as in the transfer line to the mass spectrometer cause degradation of certain explosives. The resulting spectra from GC-MS do not provide structural information and are not useful for identification. In this situation, the information gained from the GC-MS analysis is analogous to that of other universal GC detection methods and is limited because only retention data are obtained.

Other sample introduction methods have also been used to analyze explosives by MS. Direct exposure also known as direct inlet MS has been applied with reduced temperatures in the ionization source to investigate the fragmentation of explosives. This method involves introducing the sample into the ionization source using an insertion probe. Although the sample is usually introduced as a solid, liquids can be evaporated on the probe for analysis by direct exposure MS. The application of direct exposure MS is difficult for the trace analysis of explosives due to the limited amount of analyte and complexity of sample matrices.

MS instruments consist of three basic components; ion source, mass analyzer, and ion detector, which are generally operated under vacuum. The role of the ion source is to ionize the sample and focus the resultant ions into an ion beam. The mass analyzer
separates these ions based on their mass to charge ratio. The most common mass analyzers used following GC include quadrupole and ion-trap instruments. The specific type of trapping mass analyzer refers to the quadrupole ion-trap, although other types of instruments are used in research laboratories, such as linear ion-trap and ion cyclotron resonance mass spectrometers. The ion detector serves to “count” the ions exiting the mass analyzer by current amplification, such as an electron multiplier.

Several ionization techniques have been used for the analysis of explosives. Electron impact ionization (EI) is ubiquitous with GC-MS instrumentation. In these instruments, the EI source functions by supplying a cascade of electrons at 70 eV at the exit of the GC column. These high energy electrons bombard the sample resulting in either positive or negative ions depending on the polarity of the ion source. Positive ions are formed as a result of the electron bombardment removing an electron from the sample molecules. The positive ion-radical is referred to as the molecular ion. Due to the excess energy imparted on the sample molecules, the molecular ions often decompose to form fragment ions. The energy of the EI source causes many explosives to fragment. For example, the GC-MS analysis of the nitrate ester explosives, EGDN, NG, and PETN results in fragmentation to nitronium and nitrate ions.\textsuperscript{1,6} The ions correspond to mass spectral peaks at $m/z$ 46 and 62 using the positive ion mode. Because these compounds are indistinguishable using EI, the results of the GC-MS analysis of nitrate esters provides no structural information. Negative ion EI is rarely used due to the high energy imparted on the sample.
To address the decomposition of explosives and increase selectivity, chemical ionization (CI) has been used. The pressures used in EI and CI sources differ significantly ranging from $10^{-6}$ up to 1 Torr. Because EI sources provide very efficient ionization, the pressure in the source is much lower to reduce the background signal in the resulting mass spectra. In contrast, CI sources operate at relatively high pressures and depend on the type of mass analyzer. Using beam type instruments such as quadrupole mass analyzers, the CI pressures may be as high as 1 Torr. Alternatively, quadrupole ion-trap instruments require lower pressures to prevent space-charge effects. As a result of ion collisions with the bath gas, space charging occurs and causes apparent mass shifts in this type of mass analyzer. In commercial quadrupole ion-trap instruments, the mass shifts are overcome by an altered hyperbolic geometry.

CI is considered a “softer” ionization technique compared to EI. The operation of the CI source is similar to EI except that a reagent gas, also called the moderator gas is used to facilitate sample ionization. Depending on the source polarity, the reagent gas serves to dampen the high energy electrons to produce positive reagent ions or thermal electrons. Positive reagent ions are formed by electron bombardment of the reagent gas. The positive reagent ions undergo proton transfer reactions with the sample in the positive ion mode. For explosives analysis, the negative ion mode is more commonly used. In NCI mass spectrometry, the sample reacts with the thermal electrons by resonance electron capture. There are three resonance electron capture mechanisms: collisional stabilization, dissociative and non-dissociative electron capture. Because there are a limited number of molecules capable of capturing thermal electrons, the NCI
mode is highly selective and sensitive to compounds with electronegative substituents. The detection of explosives using NCI takes advantage of the inherent electronegativity of these nitrated compounds.

a. Negative Chemical Ionization Mass Spectrometry

The operation of the NCI source makes use of a reagent gas resulting in the formation of thermal electrons. The thermal electrons are captured by explosives to produce negative ions by resonance electron capture. Several different reagent gases have been used with NCI for the analysis of explosives, such as methane, isobutane, ammonia, water, hydrogen, and halogenated alkanes. Methane is the most commonly used reagent gas. The advantages of methane in NCI include its high proton affinity, which promotes the formation of deprotonated molecules and prevents neutralization reactions from occurring in the ion source. While isobutane is considered a softer reagent compared to methane, its use with explosives has been demonstrated in specific cases. A limitation of using isobutane for trace detection of explosives is the inadequate purity of the gas in a constant supply. The use of ammonia, water, hydrogen, and halogenated alkanes as the reagent gas for the analysis of explosives has been investigated to interpret reaction mechanism involved with the fragmentation of explosives.
i. Resonance Electron Capture

Explosives undergo one of three resonance electron capture mechanisms, which include collisional stabilization, dissociative and non-dissociative electron capture. The type of resonance electron capture depends on the specific compound and can be illustrated with the different classes of organic explosives. As with the positive ion mode, nitrate esters fragment using NCI. These compounds undergo dissociative electron capture to produce mainly nitrite and nitrate ions. Certain reports have demonstrated the detection of higher masses but utilized specialized sample introduction techniques, such as low temperature direct exposure probe. Nitroaromatic and nitramine compounds also capture electrons by dissociative electron capture. However, certain quantities of these compounds undergo collisional stabilization resulting in distinct fragmentation patterns. For example, NCI mass spectra of nitroaromatic compounds such as TNT typically show losses of water and nitric oxide. The nitramine compounds, which can be considered as repeating $[-\text{CH}_2\text{N(NO}_2\text{)}\text{CH}_2\text{N}^-]_x$ units ($x = 3$(RDX), 4(HMX)) undergo fragmentation illustrating the loss of 1$x$ and 2$x$ for RDX and HMX, respectively.

D. High-Performance Liquid Chromatography

HPLC has been the method of choice for the separation of nonvolatile organic explosives owing to the wide range of selectivity. Like GC methods, universal and selective detectors have been used with HPLC. Ultraviolet (UV) detection is the most commonly used detector. The HPLC-UV analysis of specific explosive compounds has
been standardized in U.S. EPA Method 8330, AOAC International Official Method 986.221, and American Society for Testing and Materials (ASTM) Method D5143-90. \(^{31,32}\) Selective detectors used with HPLC for specific explosive compounds include chemiluminescence, electrochemical, and fluorescence. \(^8\) The chemiluminescence based TEA detector and pendant mercury amperometric detection has been used for several explosive compounds. Fluorescence detection has been used for specific explosives, such as RDX and HMX. \(^{33}\) These selective detectors are limited in explosives analysis by specific drawbacks. Although the TEA detector is very selective for nitrate ester and nitramine explosives, the sensitivity is poor for nitroaromatic explosives. \(^1\) The pendant mercury electrochemical detector requires special considerations, such as extensive sample clean-up to prevent electrode fouling and removal of oxygen from the system. Fluorescence detection is limited to specific explosives, which produce decomposition products that are amendable to derivatization with a fluorophore.

1. HPLC-MS

HPLC-MS is ideal for the analysis of nonvolatile organic explosives because MS is the preferred detector for the identification of explosives. Initial HPLC-MS applications utilized thermospray ionization. \(^{34-37}\) Thermospray ionization operates by passing a liquid effluent through a heated capillary, which results in solution vaporization. This process is called vaporization with thermospray but it is also known as desolvation in atmospheric pressure ionization techniques. In thermospray MS, the
vaporized effluent passes through a nozzle that leads to the mass analyzer. Like the limitations in GC-MS methods, thermospray is inadequate for sample identification of certain compounds due to the thermal degradation. More recent advances in the analysis of explosives by HPLC-MS make use of atmospheric pressure ionization techniques. Both atmospheric pressure chemical ionization (APCI) and ESIMS have been used in several research studies on the analysis of explosives. The ionization sources in these methods are held at atmospheric pressure as opposed to evacuated ionization sources used with GC methods. The commercially available atmospheric pressure ionization MS instruments make use of nitrogen as the nebulizing and bath gases in the ionization sources. While APCI is similar to thermospray with the high temperature capillary, a corona discharge needle is placed at the capillary exit, through which a current is passed to facilitate ion formation. APCI is a mass dependent technique that produces ions by corona discharge. By using the mobile phase as the chemical ionization reagent along with the bath gas, APCI generates gas-phase analyte ions via ion-molecule reactions.

The use of APCI-MS for the analysis of explosives requires that low temperatures be used within the ionization source to prevent fragmentation during the desolvation process. Typically, APCI-MS is used in the negative ion mode for explosives analysis. Despite quite good sensitivity for the trace level detection of nonvolatile explosives such as PETN and RDX, the use of APCI is limited with respect to the analysis of more volatile compounds such as EGDN and NG.

A series of explosives were detected as chloride adducts using HPLC-APCI-MS, with detection limits ranging from 10-60 ppb. These studies used low
concentrations of dichloromethane, chloroform and carbon tetrachloride additives in the mobile phase to supply chloride for adduct formation. Another study used these chlorinated solvents as well as ammonium chloride for the analysis of nitrate ester explosives by HPLC-APCI-MS. The authors also investigated the formation of various adducts with nitrate esters using HPLC-ESIMS. In addition to chloride, nitrite, nitrate, and propionate adducts were used for the detection of the nitrate esters including EGDN, NG, PETN, and mono- and dinitrated alcohols of butane and propane.

2. Electrospray Ionization Mass Spectrometry

ESIMS is an emerging analytical method originally discovered by Dole in 1968. The ESIMS analysis of large biomolecules made possible by Fenn and coworkers was a prominent contribution that has popularized this mass spectrometric technique. ESIMS can also be used for the analysis of small molecules such as explosives. ESIMS is a concentration dependent technique that produces analyte ions from a liquid solution. By passing the liquid through a capillary held at a high potential with respect to a counter electrode, gas-phase ions are produced from charged droplets. A schematic of a Bruker Esquire-LC system (Bruker Daltonics, Bremen, Germany) electrospray ionization source with a quadrupole ion-trap mass spectrometer (QITMS) ESIMS instrument is shown in Figure 2.2.

Two major processes occur in the electrospray ionization source: charged droplets are produced at the capillary tip and gas-phase ions are produced from the droplets.
Because the liquid solution contains electrolytes, a current passes between the capillary and counter electrode. This principle has been used to describe the electrospray ionization source as a special type of a controlled-current electrolytic flow cell. Due to the potential applied to the capillary, an electric field is produced across the flow cell, which results in an excess charge that is retained by the droplets emitted from the capillary. The excess charge is carried by the droplets through a small orifice in the counter electrode that serves as the entrance to the mass spectrometer. The ESIMS mass spectrometer consists of a desolvation region, ion transport section, mass analyzer, and detector. As shown in Figure 2.2, the desolvation capillary and skimmers comprise the desolvation region and the ion transport region includes the octapole and lenses, which is followed by the QITMS and detector.

The production of gas-phase ions by electrospray ionization has been described by two conventional mechanisms: charged residue model (CRM) and ion evaporation theory (IET). The CRM proposes that the droplet fissioning process called Coulomb fission gives rise to smaller charged droplets that subsequently undergo their own fissioning process at or “just prior to” the Rayleigh stability limit. The Rayleigh stability limit is defined as the point at which the force of the electrostatic repulsion on the droplet surface equals the surface tension of the solvent. Solvent evaporation promotes droplet fissioning as a result of the increasing surface charge to volume ratio.
The Coulomb fission process increases the droplet charge density because most of the excess charge produced by ESI is maintained as smaller “offspring” droplets are produced. The fissioning process continues until the remaining particles contain only a single charged species. The CRM has been shown to be responsible for the production of large multiply charged ions, such as proteins.
The IET postulates that during the droplet desolvation process, the surface charge density increases until coulumbic repulsion exceeds the surface tension of the solvent, which results in the production of charged particles directly from the droplet surface.\textsuperscript{47} As the droplet size decreases to a specific stage, IET suggests that ion production occurs preceding the Rayleigh stability limit. Specifically, when the electrostatic repulsion on the droplet surface is high enough to overcome ion adhesion, solvated analyte ions are converted to gas-phase ions by evaporating directly from the droplet. In previous experiments addressing the mechanism of ESIMS, two separate research groups postulated IET was responsible for the production of ions from samples containing alkyl ammonium and alkali halide compounds.

A third mechanism, known as the equilibrium partitioning model is similar to the IET in that ions are generated directly from the droplet surface.\textsuperscript{51} The equilibrium partitioning model differs with the assumption that ions partition between the charged exterior and neutral interior of the droplet, which exist as two distinct phases. According to the equilibrium partitioning model, the ions observed in the mass spectrum depend upon several solution parameters, such as the polarities of the solvent and solute ion. In the equilibrium partitioning model, polar solvents will promote the partitioning of polar solutes to the surface and enhance their ESIMS intensity. Alternatively, neutral nonpolar solvents will restrict the partitioning of nonpolar solutes to the surface and thus decrease their ESIMS signal. Because the equilibrium partitioning model is similar to the IET in that ions are generated directly from the droplet surface as the droplet size decreases, polar solvents induce the partitioning of nonpolar solutes toward the droplet surface.
These principles have been used to relate ESIMS response to the nonpolar character of the side chain groups in small peptides. This relationship was extended to correlate the ESIMS response of small peptides to their retention characteristics in reversed-phase HPLC demonstrating increased intensity for more hydrophobic molecules.

a. Positive Ion ESIMS

The operation of the ESIMS source makes use of a capillary held at a high potential with respect to a counter electrode to produce gas-phase ions from charged droplets. In the positive ion mode, the capillary potential is positive and the counter electrode is typically held at or just below ground potential. The sample is pumped through the capillary resulting in a laminar flow profile that serves to renew the solution reaching the capillary tip. Because there is such a large potential difference between the capillary and the counter electrode, cations within the solution migrate away from its surface and anions migrate toward the surface. As a result of the migrating ions along with the laminar flow, the solution is pushed out of the capillary tip. The electrosprayed solution contains electrolytes to maintain the microampere level current that passes between the capillary and counter electrode. The electrolytes are deliberately added or may be present as impurities in the solvent. Electrolytes also promote the formation of excess charge on the droplets. As a result, positive ions are produced by the electrospray process and subjected to mass analysis for detection.
Positive ion ESIMS is typically used with basic compounds that are detected as protonated molecules. Most nitrated organic explosives are not easily protonated by the electrospray process. For this reason, the use of positive ion ESIMS has been limited in the analysis of explosives to specific compounds such as smokeless powder additives and ammonium nitrate (AN). HPLC-ESIMS was used for the analysis of methyl centralite (MC) in a single smokeless powder from gunshot residue (GSR). Positive ion ESIMS was also employed without chromatographic separation to quantify DPA and its nitrated derivatives from GSR. The characterization of AN was demonstrated by Zhao and Yinon. In this study, adducts of AN were detected in both positive and negative ion mode at different temperatures over a range of m/z 50-500. While the study suggests that temperatures below 150 °C result in enhanced detection of adduct ions between m/z 100-300, there was no discussion on the principles by which these adduct ions are formed. Additionally, the capability of positive ion ESIMS to discriminate among different sources of AN was not provided.

Although positive ion ESIMS has been used for the analysis of certain explosive samples, the important factors to be considered for optimal detection conditions have not been directly addressed in forensic applications. The selection of the optimal detection parameters involves consideration of analyte characteristics, instrument operation, and in regard to HPLC, the mobile phase composition. In HPLC-ESIMS applications, the organic modifier is used to facilitate chromatographic resolution and the electrolyte is used to promote ionization. The choice of the organic modifier and electrolyte used in method development relate to solution parameters that influence the ESIMS signal.
The physical properties of the mobile phase solution, such as volatility, surface tension, viscosity, conductivity, and dielectric constant affect the production of ions by electrospray.\textsuperscript{60} The resulting solution parameters are associated with the mechanisms by which ions are produced during the ionization process. The physical properties of the analyte are also of great importance in determining the optimal ESIMS parameters. The important instrumental factors to optimize include ionization potential, interface temperature, and nebulizing gas flow rate. The instrumental parameters are analyte dependent and thus cannot be as readily modified as the mobile phase composition. The selection of the mobile phase composition has been rationalized with respect to the optimal chromatographic resolution with minimal analysis time and superior method sensitivity. Due to the qualitative requirements in forensic applications, the mobile phase composition for optimal ESIMS detection is secondary to chromatographic resolution and analysis time. For example, previous studies on the analysis of smokeless powder components by HPLC-ESIMS and tandem ESIMS in the positive ion mode did not report the use of mobile phase components as electrolytes to promote ionization.\textsuperscript{54,55,61} Other investigations including negative ion ESIMS studies used ammonium acetate at arbitrarily chosen concentrations.\textsuperscript{9,62-65} Additionally, gradient elution HPLC is often applied without taking into account changes in the mobile phase composition and their effects on the ESIMS intensity. The solution parameters inherently change during chromatographic run, which can affect the ESIMS response. The changes in the ESIMS response as a function of the gradient mobile phase composition have not been well characterized in forensic applications.\textsuperscript{66}
b. Negative Ion ESIMS

The number of applications using negative ion ESIMS for the analysis of organic compounds and biomolecules are considerably less than those involving the positive ion mode. Negative ion ESIMS is commonly used for the analysis of acidic compounds. Because the capillary is held at a negative potential, gas-phase anions are released from the tip and focused into the mass spectrometer. The formation of negative ions in the ESIMS source can be attributed to two main processes: deprotonation and adduct ion formation. The basis of electrospray ionization was illustrated in the earliest reports of ESIMS using these two mechanisms.

The deprotonation of acidic compounds is the prominent mechanism in negative ion ESIMS. Neutral compounds without acidic groups that are not easily deprotonated can also be detected. These neutral analyte molecules form adduct species through non-covalent gas-phase interactions with anions such as chloride and iodide. The relative intensity of the anionic adducts has been attributed to the gas-phase basicities of the deprotonated molecule and the anion. When the deprotonated analyte molecule and the anion have comparable gas-phase basicities, the relative intensity of the adduct ion increases compared to the intensity of the deprotonated species alone. The formation of anionic adducts requires the use of anions that may be added as electrolytes. While electrolytes are useful in promoting the formation of excess charge on the droplets, the solution conductivity must be controlled to prevent electrical discharge at the electrospray capillary. This process, also known as corona discharge, is caused by an
increased current between the capillary and the counter electrode resulting in electrons being field-emitted from the capillary. Because the electrospray source is a controlled-current electrolytic device, electrical discharge occurs because the current is no longer constrained by the gap resistance between the droplets and surrounding gas along the path from the capillary to the counter electrode. The excess current is commonly observed when using highly conductive aqueous solutions. While electrical discharge causes analytes to dissociate or fragment, this process can be reduced by decreasing the solution conductivity or using an electron scavenger. For example, Yamishita and Fenn reported the use of O$_2$ in the ionization source to prevent the onset of electrical discharge.\(^{41}\)

Negative ion ESIMS has a number of advantages for the analysis of explosives.\(^{7}\) While the use of negative ion ESIMS has been promoted by the capability of explosives to capture electrons, these energetic compounds are actually detected as a result of deprotonation and adduct ion formation.\(^{11,13}\) The favored process depends on the stability of the negative ions that are subsequently detected in the mass spectrometer. With the exception of picric acid (2,4,6-trinitrophenol), nitrated explosives do not contain ionizable protons amendable to negative ion formation by deprotonation. However, electrospray is a soft ionization technique that involves important gas-phase interactions. The gas-phase basicity is the important factor in the deprotonation of neutral compounds without acidic protons. The deprotonation of these compounds is a result of lower gas-phase basicity than other more acidic species in the electrosprayed solution. For example, the gas-phase basicity of TNT ($\Delta G_f = 1351 \pm 21$ kJ/mol) is lower than
chloride (1374 ± 8.4), nitrate (1396 ± 1.3), and acetate (1429 ± 8.4) and has primarily been detected as a deprotonated molecule.\textsuperscript{10,11,70,71} When the gas-phase basicities of the deprotonated analyte molecule ([M-H]⁻) and the anionic species ([A]⁻) are comparable, the detection of the adduct ion is favored. Thus, the adduct ion can be considered a complex consisting of two gas-phase negative ions in equilibrium with a proton that has been illustrated as “[M-H]⁻····H⁺····[A]⁻”.\textsuperscript{67-69} For instance, HMX has been detected as an acetate adduct.\textsuperscript{62,70} Although the gas-phase basicity of HMX has not been reported, it is expected to be comparable to acetate.\textsuperscript{71}

Several researchers have reported the use of HPLC-ESIMS and tandem MS for the detection of high explosives in the negative ion mode.\textsuperscript{9,13,28,62,70,72} An HPLC-ESIMS was developed for the analysis of high explosives using the negative ion mode.\textsuperscript{9,70} This study demonstrated the detection of deprotonated molecules and adduct ions using mixtures of EGDN, NG, DNT, TNT, RDX, PETN, and HMX. The detection and fragmentation products of several high explosives were reported using ESIMS/MS.\textsuperscript{11} In this study, EGDN, NG, TNT, tetryl, RDX, and HMX were detected as deprotonated molecules in an ion-trap mass spectrometer. The use of this detector allowed for tandem MS studies using daughter-ion, parent-ion and neutral loss scans for the characterization of the explosives. While a variety of adduct species were shown in the reported spectra for NG, tetryl, RDX, and HMX, these adduct ions were not characterized by tandem MS. In another study, selected explosives were used to characterize fragile negative ions in the ion-trap mass spectrometer.\textsuperscript{28} Because fragile ions such as the deprotonated molecule of HMX prematurely dissociate during the normal operation of the ion-trap, mass spectral
peaks are wider than expected and thus affect resolution. The HPLC-ESIMS analysis of selected high explosives was compared with results from GC-MS and HPLC/UV following aqueous extraction of explosive residue. Although no spectra were reported for EGDN, NG, TNT, RDX, and PETN, Thompson and coworkers reported the use of ammonium nitrate as the electrolyte to promote ionization and adduct formation. The composition of explosives were characterized from \( m/z \) 100-1000 using Fourier transform ion cyclotron resonance mass spectrometry in selected forensic samples. While this study illustrated the high resolving power of this technique, the results were restricted to the detection of NG, TNT, RDX, HMX, and PETN in a limited number of samples. In a more recent study, various adducts of several nitrate esters were characterized by negative ion ESIMS. Nitrate, nitrite, propionate, and chloride adducts of selected nitrate ester explosives including EGDN, NG, PETN, mono- and dinitronitroglycerin were detected by ESIMS and APCI in the negative ion mode. The results of this study indicated that negative ion ESIMS was more sensitive than APCI for the detection of the selected adduct ions.
III. Chapter 3. Experimental Procedures

A. Introduction

The analysis of explosives was performed using gas and liquid chromatography and mass spectrometry methods including gradient reversed-phase HPLC-ESIMS in the positive ion mode, isocratic reversed-phase HPLC-ESIMS in the negative ion mode, GC/ECD, and GC-MS. The optimization of the ESIMS detection parameters was performed by direct liquid introduction (DLI) prior to developing the HPLC methods. Gradient reversed-phase HPLC-ESIMS in the positive ion mode was used for the analysis of smokeless powder additives. GC/ECD and GC-MS methods were developed for the environmental survey of explosives residue in public places. The separation and detection of high explosives was performed using isocratic reversed-phase HPLC-ESIMS in the negative ion mode.

B. Smokeless Powder Analysis

Two gradient reversed-phase HPLC-ESIMS methods with positive ion detection were developed for the analysis of smokeless powder additives. Initial parameters for the detection of smokeless powder additives were determined using DLI with a flow rate of 0.02 mL/min. in the positive ion mode. A gradient reversed-phase HPLC method was developed with the UV detector and positive ion ESIMS. This novel method was used for the analysis of several unburned smokeless powders. Subsequent studies for the characterization of several solution parameters associated with gradient elution method were performed by DLI to enhance the ionization efficiency of powder constituents. The
solution parameters evaluated included electrolyte concentration, mobile phase organic
modifier content, and mobile phase pH. A second HPLC-ESIMS method was validated
and used for the analysis of different unburned smokeless powders and samples from
pipe bombs filled with these powders.

1. Chemicals

Ammonium acetate (CH₃COONH₄) and HPLC grade solvents, methylene chloride (CH₂Cl₂) and methanol (CH₃OH), were obtained from Fisher Scientific (Pittsburgh, PA). Milli-Q purified water was used throughout the experimental procedures. Analytical standards: N-nitrosodiphenylamine (NsDPA) (Fluka, Milwaukee, WI), diphenylamine (DPA), 4-nitrosodiphenylamine (4sDPA), 2-nitrodiphenylamine (2NDPA), 4-nitrodiphenylamine (4NDPA), 4-4′-dinitrodiphenylamine (44DNDPA), 2-4′-dinitrodiphenylamine (24DNDPA), methyl centralite (MC), ethyl centralite (EC), dimethylphthalate (DMP), diethylphthalate (DEP), and dibutylphthalate (DBP) (Acros, NJ) were prepared in methanol as 0.5 or 1 mg/mL stock solutions and stored at 4°C.

2. Instrumentation

a. Positive Ion Electrospray Ionization Mass Spectrometry

A Bruker Esquire-LC system (Bruker Daltonics, Bremen, Germany) electrospray ionization source with a quadrupole ion-trap mass spectrometer (QITMS) was used with two sample introduction methods: DLI and HPLC. A Cole-Parmer Syringe Infusion
Pump (Cole-Parmer, Vernon Hills, IL) was used for DLI at flow rates between 0.005 to 0.02 mL/min. Bruker Esquire Instrument Control software (3.1) was used to set-up the ESIMS source, ion-optics, and QITMS settings. The ESIMS source settings were: ESI needle (4 kV), capillary temperature (250 °C), N₂ nebulizing gas (40 psi), N₂ drying gas (5 L/min.). The nitrogen gas was generated in the laboratory using a Model 75-72 Parker-Balston nitrogen generator (Parker Hannifin Corp, Filtration and Separation Division, Tewksbury, MA). The ion optics were: capillary offset voltage (35 V), skimmer 1 (25 V), skimmer 2 (6 V), octapole (2.4 V), lens 1 (-5 V) and lens 2 (-60V). The QITMS was used for positive ion detection from m/z 30-500.

b. Gradient Reversed-Phase HPLC-ESIMS

A Hewlett-Packard 1100 HPLC system (Agilent, Palo Alto, CA) was used for the analysis of smokeless powders with a variable wavelength UV detector at 230nm that was coupled to the Bruker Esquire-LC system ESIMS. The HPLC system was controlled by Chemstation software (A.06.03) that is linked to the Bruker Esquire Instrument Control software to apply the ESIMS parameters listed above. A Restek (Restek Corporation, Bellefonte, PA) Pinnacle octyl column (C₈), 2.1 × 100 mm, 3 μm particle size, and 120 Å average pore size was used at room temperature. The two gradient reversed-phase HPLC-ESIMS methods were performed using different mobile phase compositions. In both methods, the solvents were degassed and filtered by the HPLC system. The mobile phase solvents were passed through a solvent inlet filter (Agilent Part
No. 5041-2168) and vacuum degasser prior to the binary pump and then passed through a metal free in-line filter (Alltech Part No. 68250). The mobile phase in the first method consisted of 50\% methanol/50\% aqueous 1 mM ammonium acetate that was programmed to 95\% methanol/5\% aqueous 1 mM ammonium acetate in 25 min. The second method made use of the same linear gradient from 50-95\% methanol but a constant 2 mM ammonium acetate concentration was used in both the methanol and the aqueous fractions of the mobile phase.

3. Sample Preparation

a. Unburned Powders

The smokeless powders samples were selected from various manufacturers including IMR (Plattsburgh, NY), Accurate Arms Company (McEwen, TN), Hodgden (Shawnee Mission, KS), and Alliant (Radford, VA). The smokeless powder sample preparation method developed for GC-MS analysis was adapted for use with HPLC-UV.\textsuperscript{20,23} Individual smokeless powders samples were prepared by extracting 5 mg of the unburned powder with 250 µL methylene chloride overnight. A 20 µL aliquot was removed into a clean vial and evaporated under a stream of nitrogen gas. The samples were reconstituted with 40 µL methanol. An injection volume of 5 µL was used for the quantitative HPLC-ESIMS analysis.
b. Pipe Bomb Samples

Pipe bombs were constructed by Ohio State Fire Marshall’s forensic laboratory personnel. The pipes were plastic polyvinyl-chloride pipes 1 inch in diameter with plastic endcaps. Twelve different powders from various manufacturers were used as fillers. These powders were also analyzed as unburned samples and consisted of four groups from different manufacturers with at least two different lots of powders. The pipe bombs were ignited by electric blasting caps inside a specially constructed cage to contain the pipe fragments. The cage consisted of a cardboard box to hold the pipe bomb, a large tractor tire with a wire grating on top and clean sand covering the ground below.

Cotton swabs were used for sampling the surfaces inside the cardboard box for each pipe bomb. The swabs were stored in sealed plastic bags for transport to the laboratory. The cotton swabs were divided into four parts with at least two portions containing the visible residue. One of the quartered pieces was placed in a clean glass vial for each pipe bomb residue sample. Using 3 mL methylene chloride the samples were extracted for 72 hours. Using 5 mL plastic disposable syringes, the methylene chloride extract was recovered from each cotton swab and stored in a small glass test tube. A 600 µL portion of the extract was placed in a 2.5 mL sample vial and blown to dryness with nitrogen gas. Samples were reconstituted with 20 µL of a 50% methanol/water mixture and 5 µL was used for the autosampler injection volume. The percent composition of the smokeless powder in the residue was estimated based on the sample preparation procedure for the unburned powders.
C. Survey of Environmental Background Interferences Affecting Explosive Residue Analysis

This project was planned for the forensic analysis of trace organic explosives in public places and arranged in several stages. The portion of the project included in this research involved methods development for the separation and detection of six high explosives using two GC systems with selective detectors. Methods using GC/ECD and GC-MS with methane NCI were validated for sample screening and confirmation, respectively.

1. Chemicals

HPLC grade solvents, acetone (CH$_3$COCH$_3$), methanol (CH$_3$OH), and isopropanol ((CH$_3$)$_2$HCOH) were obtained from Fisher Scientific (Pittsburgh, PA). The explosives used in this study were acquired from Cerilliant Corporation (Round Rock, TX) with at least 98% or greater purity and include EGDN (ethylene glycol dinitrate), NG (nitroglycerin), TNT (trinitrotoluene), PETN (pentaerythritol tetranitrate), RDX (cyclo-1,3,5-trimethylene-2,4,6-trinitramine), and HMX (cyclo-1,3,5,7-tetramethylene-2,4,6,8-tetranitramine). Helium carrier gas (Ultra-High Purity) was obtained from Air Products and Chemicals, Inc. (Allentown, PA) and used in both GC/ECD and GC-MS instruments. Nitrogen gas (N$_2$) (High Purity, Air Products and Chemicals, Inc.) was used
as the anode and make-up gases in the ECD. Methane (CH₄) chemical ionization reagent gas was used in the GC-MS for NCI detection.

2. Instrumentation

a. Gas Chromatography/Electron Capture Detection

A 6890 Hewlett-Packard (Agilent) GC/ECD system with splitless injection, helium carrier gas, and a large bore HP-5 (DB5 type, 95% dimethyl 5% diphenyl polysiloxane) column 10 m length, 0.53 mm inner diameter, 2.65 μm film thickness was used for the detection of high explosives. A temperature program of 50 °C for 1 min. to 150 °C at 20 °C/min., to 250 °C at 40 °C/min. was used with a carrier gas flow rate of 35 mL/min. A detector temperature of 250 °C was employed with N₂ make-up gas at a flow rate of 60 mL/min., which was also used as the anode gas.

b. Gas Chromatography-Mass Spectrometry

A Finnigan GCQ GC-MS controlled by Finnigan XCalibur (Ver. 1.1) software was equipped with a Chrompak CP8MS column (DB5MS type), 12 m length, 0.25 mm inner diameter, 0.25 μm film thickness and detection with methane chemical ionization in the negative ion mode. The GCQ instrument was used in the splitless mode, 180 °C injector temperature, 200 °C transfer line temperature, He carrier at 80 cm/s, and oven temperature program of 50 to 200 °C at 12 °C/min. The analytical column was used as
the transfer line between the GC oven and mass spectrometer. The QITMS mass spectrometer was used with an ion source temperature of 150 °C and methane CI gas pressure of 60 mTorr in the ionization source at a foreline pressure of 90 mTorr.

The Finnigan GCQ GCMS was also equipped with a Restek Rtx5MS (DB5MS type), 15 m length, 0.53 mm inner diameter, 1.5 µm film thickness column and 0.1 mm fused silica transfer line. The same parameters were used for the injector, GC oven program, and mass spectrometer but the carrier gas flow was set to 90 cm/s. The effluent was split in the GC oven by sliding the analytical column over the transfer line, thus venting part of the flow into the GC oven. The transfer line was constructed from an untreated section of fused silica with a 0.1 mm inner diameter, 0.325 mm outer diameter and 50-90 mm in length. The effect of the transfer line length inside the oven was tested by evaluating the peak shapes and intensities of explosive standards. While the mass spectrometer was vented, the transfer line was placed between the GC oven and detector at a total length of ~90 mm. Because a length of ~40 mm is required for proper installation, the analytical column was initially fitted over the 50 mm length inside the oven. Without venting the mass spectrometer, sections of the transfer line inside the oven were removed in 10mm increments from 50 mm down to 10 mm to evaluate the response of the explosives.
3. Sample Preparation

The GC/ECD and GC-MS methods were developed and validated using explosives standards in acetone. Because dry cotton swabs will be used for sampling in public places, an evaluation of the extraction procedure was used to assess the recovery and detection of the selected explosives. Dry cotton swabs were used to determine the recovery of the explosives by extracting standards with acetone followed by sample screening using GC/ECD. Cotton swabs were cleaned in individual glass vials by rinsing and vigorously shaking with 3 washes of deionized, distilled water followed by 3 washes with isopropanol. The swabs were then removed from the vials and placed on a clean watch glass and dried over night at 70 °C. After drying, the cotton was cut into four smaller pieces and placed into clean vials.

a. Cotton Swab Samples

Using clean metal forceps, a cotton swab was removed from the glass vial and spiked directly with an appropriate amount of standard. The swab was inserted into a disposable plastic syringe. An Anotop 0.2 μm filter was attached to the end of each syringe, and 3 mL of HPLC grade acetone was added. The cotton swab and acetone were allowed to remain in the syringe for 30 seconds before the plunger was inserted and acetone was pushed into a glass test tube. The acetone extract was either injected into the GC/ECD or blown down with nitrogen gas to concentrate dilute samples.
D. Detection of Explosives and Related Compounds Using ESIMS in the Negative Ion Mode

The detection of high explosives was performed using ESIMS in the negative ion mode. Initial ESIMS parameters for the detection of explosives were determined using DLI with flow rates ranging from 0.005 to 0.02 mL/min. Several detection parameters were evaluated by using DLI including anion concentration, mobile phase pH, and ESIMS operating temperature. An isocratic reversed-phase HPLC-ESIMS method was developed to evaluate the analysis of high explosives.

1. Chemicals

Ammonium acetate (CH$_3$COONH$_4$), ammonium formate (CHOONH$_4$), ammonium nitrate (NH$_4$NO$_3$), and ammonium chloride (NH$_4$Cl) and HPLC grade solvents: methanol (MeOH), acetonitrile (ACN), and isopropanol (IPA), were obtained from Fisher Scientific (Pittsburgh, PA). Analytical standards of the explosives were prepared in different solvent mixtures using Milli-Q purified water throughout the experimental procedures. The compounds used in this study included EGDN, NG, TNT, PETN, RDX, and HMX obtained from Cerilliant Corporation (Round Rock, TX).

2. Instrumentation

a. Negative Ion Electrospray Ionization Mass Spectrometry

A Bruker Esquire-LC system electrospray ionization source with a QITMS was used to evaluate the ionization and tandem MS detection of the selected explosives in the
negative ion mode. A Cole-Parmer Syringe Infusion Pump (Cole-Parmer, Vernon Hills, IL) was used for DLI at a flow rate of 0.004 to 0.01 mL/min. The ESIMS source settings were: ESI needle (-4 kV), capillary temperature (80-200 °C), N₂ nebulizing gas (5-25 psi), N₂ drying gas (1-5 L/min.). The ion optics were: capillary offset voltage (10 V), skimmer 1 (8 V), skimmer 2 (6 V), octapole (2.4 V), lens 1 (5 V) and lens 2 (30 V). The QITMS was used for negative ion detection from \( m/z \) 30-500.

b. Isocratic Reversed-Phase HPLC-ESIMS

An SSI Model 222D HPLC pump (Shimadzu Scientific Instruments, Inc., Kyoto, Japan) was employed at 0.15 mL/min. The detection of the explosives was accomplished by negative ion ESIMS using the parameters above. A Hewlett-Packard C\(_{18}\) column (2.1 × 100 mm) was used with two different isocratic mobile phases consisting of 50% MeOH/50% aqueous mixtures for the comparison of two instrumental arrangements. These mixtures were purged with helium for 10 min. and not filtered prior to use as mobile phases. The first method was applied with a mobile phase of 50% MeOH/50% water for HPLC separation with the post column addition of a 50% MeOH/50% aqueous solution containing 0.5 mM ammonium nitrate and 1 mM ammonium chloride. The post column addition was performed by DLI at 0.015 mL/min. into a tee union after the HPLC column. This post column arrangement resulted in a solution of 50% MeOH/50% aqueous 0.05 mM ammonium nitrate and 0.1 mM ammonium chloride at the ESIMS source. The second instrumental set-up was used with a mobile phase containing 50% MeOH/50% aqueous 0.05 mM ammonium nitrate and 0.1 mM ammonium salts of
formate, acetate and chloride throughout the HPLC-ESIMS system. A Hewlett-Packard 1100 HPLC system (Agilent, Palo Alto, CA) autosampler was used for the sample introduction of 10 µL.

E. Data Analysis

Microsoft Excel (Ver. 10, 2002) was used to tabulate data obtained from the instrumental software packages including Agilent Chemstation, Finnigan XCalibur, and Bruker Esquire Data Analysis. Excel, Minitab (Ver. 13.31), and Matlab (Student Ver., PLS Toolbox) software were used for the calculation of the linear regression, quantitative analysis, limits of detection, and analysis of variance (ANOVA) data. All statistical evaluations were performed at the 95% confidence level.

The linear regression calibration parameters were computed in Excel using the LINEST function. The limits of detection were calculated using 3 times the standard error of the y (Intensity) estimate and transformed to concentration units. The confidence intervals for the limits of detection were computed using the standard error of the intercept in concentration units.
IV. Chapter 4. Development of Gradient Reversed-Phase HPLC-ESIMS Methods for the Analysis of Smokeless Powder Additives

A. Introduction

Smokeless powders are manufactured for optimum propellant performance for small arms ammunition. Due to different propellant applications and reformulation, there is a wide range of compositional differences between commercially available smokeless powders. These unique differences in smokeless powder composition allow for the determination of a distinguishable chemical profile.\textsuperscript{1,8,22,32} The forensic analysis of smokeless powders is carried out by identifying and quantifying the organic components using instrumental techniques. GC and HPLC have been used with various detection modes for the analysis of organic additives in smokeless powders.\textsuperscript{1,8,20-22,74} Mass spectrometry has been employed for the detection of the organic constituents in smokeless powders. For example, Martz and Laswell used GC-MS results and morphological properties to differentiate smokeless powders.\textsuperscript{20} While GC methods are generally used, a major disadvantage is the thermal degradation of certain powder components. HPLC methods can be used given that the analysis takes place at room temperature. Due to the wide range of polarity of the components and difficulty in separating geometrical isomers, most HPLC methods are limited to the analysis of certain organic additives.\textsuperscript{7,32,54,55} Reversed-phase HPLC methods with MS detection have also been employed. HPLC-thermospray ionization MS was used to detect DPA and NG in residue from pipe bombs loaded with smokeless powder.\textsuperscript{7} HPLC-ESIMS was used for the analysis of MC as well as DPA and its nitrated derivatives in a single smokeless powder
from GSR.\textsuperscript{54,55} Despite the success in demonstrating the detection of organic smokeless powder additives from GSR, the previous ESIMS studies were limited in the number of additives detected and in the variety of smokeless powders tested.

As a result of the disadvantages in particular analysis techniques, such as thermal degradation in GC-MS and the limited number of components determined by HPLC methods, alternative methods have been developed. A gradient reversed-phase HPLC-UV method was developed for the analysis of the organic constituents in smokeless powders.\textsuperscript{23} In the present study, this gradient reversed-phase HPLC method was modified to facilitate detection by ESIMS in the positive ion mode. The gradient reversed-phase HPLC-ESIMS method was developed to establish a more comprehensive method of determination for organic powder constituents and to differentiate unburned smokeless powders by identifying and quantifying particular additives.

**B. Method Development**

The reversed-phase gradient separation method developed by Wissinger and McCord for smokeless powders analysis was adapted for ESIMS detection.\textsuperscript{23} The ESIMS detection parameters were optimized for the detection of selected powder additives as protonated molecules in the positive ion mode. The parameters were also optimized to reduce the collision-induced dissociation (CID) of dibutylphthalate in the electrospray source.\textsuperscript{75} The gradient reversed-phase separation method was modified for ESIMS detection by reducing the column size, flow rate, and adding ammonium acetate to the aqueous portion of the mobile phase. Using the optimal ESIMS parameters, the method
was used for the determination of diphenylamine and centralite based stabilizers in addition to dialkylphthalate plasticizers in several unburned powders.

C. Detection of Protonated Molecules by Positive Ion ESIMS

The Bruker Esquire-LC system ESIMS with a QITMS was used for the analysis of smokeless powder additives. Because several powder additives are basic compounds, the positive ionization mode was selected for detection. The initial detection parameters were established prior to full HPLC-ESIMS analysis. The initial ESIMS parameters were determined by DLI using analytical standards at 100 µg/mL at 0.05 mL/min. Individual standards containing 100 µg/mL DPA, 4sDPA, 4NDPA, MC, EC, and DBP were infused in 50% methanol/1 mM aqueous ammonium acetate to establish maximum electrospray ionization efficiency. The “in-source” CID of DBP, a 1,2-benzenedicarboxylic acid ester, M, 278, was discovered during preliminary experiments by the dominance of the characteristic fragmentation product ions at m/z 149 and m/z 205 in the mass spectrum. “In-source” or “up-front” CID is a molecular fragmentation process, which occurs as ions are transferred from the atmospheric pressure source to the mass analyzer. During ion transfer, the resulting energy from significant pressure and voltage differences between the capillary exit electrode “nozzle” and skimmer causes fragmentation. The type of atmospheric pressure ionization interface is an important consideration for diagnosing in-source CID. In the Bruker-Esquire instrument, the capillary length is approximately 10 cm. As opposed to interfaces with only a cone or dual capillaries, the pressure
differential between the atmospheric source and the reduced pressure region of the skimmers in the Bruker-Esquire ESIMS is dispersed over a large volume. Therefore, the voltage offset, between the capillary exit electrode and skimmer 1, was considered to be the prominent factor contributing to the CID of dibutylphthalate. The nozzle-skimmer voltage offset was reduced from 60 V to 10 V to maximize the intensity of protonated molecule of dibutylphthalate, \( m/z \) 279, and thus reducing “in-source” CID.

D. Smokeless Powder Differentiation

1. HPLC Optimization

   The development of the HPLC method was performed using the optimized ESIMS parameters for the separation and detection of the selected smokeless powder additives. While Wissinger and McCord employed a column diameter of 4.6 mm with a 1.0 mL/min. flow rate, the present method was developed for ESIMS detection with a 2.1 mm column diameter.\(^{23}\) A flow rate of 0.25 mL/min. was used to minimize peak broadening between the UV flow cell and ESIMS in the coupled detection system. An additional consideration for method development in this study was the particle size of the smaller column. Because the 2.1 mm column contained 3\( \mu \)m particles, compared to a 5 \( \mu \)m particle size used by Wissinger and McCord, a shorter column length was used to achieve efficient separation.\(^{23}\)
2. Method Validation

Daily, replicate chromatographic injections of standard mixtures with concentration range from 0.05 to 20 \( \mu g/mL \) were used to determine the precision of the HPLC-ESIMS method. The results were obtained using extracted ion chromatogram (EIC) peak areas. The repeatability for all standards ranged from 2.3 to 6.1\% for intra-assay and 1.6 to 8.4\% for inter-assay precision given in Table 4.1. The method calibration was established using linear regression data for the EIC peak areas of each component over the concentration range of 0.05 to 20 \( \mu g/mL \). To illustrate the precision of the method, the intra-assay (daily) and inter-assay (day-to-day) variation is given in Table 4.1 for the EIC peak areas of the 1.0 \( \mu g/mL \) \((n \geq 3)\) analytical standard. The inter-assay precision is slightly greater than the intra-assay precision as expected with the exception of NsDPA and MC. The differences in the variation were not significant, which is beneficial for the simultaneous determination of smokeless powder additives.

3. Quantitative Analysis

The quantitative analysis of eleven different unburned smokeless powders was performed using the HPLC-ESIMS method. Table 4.2 shows the percent composition and standard deviation for the components detected. DPA, NsDPA, and one isomer of nitrodiphenylamine were detected in each of the eleven powders. DPA is a stabilizer that is added to smokeless powder to prevent the autocatalytic degradation of NC in the
presence of moisture. Nitrous and nitric acids produced during powder aging react with DPA, which results in the formation of nitroso and nitro derivatives of DPA, such as NsDPA and 4NDPA. For example, the extracted ion chromatograms of Red Dot 900 are shown in Figure 4.1, in which DPA, NsDPA, 2NDPA, EC, and DBP were detected. Red Dot 900 was the only smokeless powder in which 2NDPA was detected without the 4NDPA isomer as illustrated in Table 4.2 and Figure 4.1. The presence of 2NDPA alone is unique given that the reactivity trend toward nitro derivative formation has been shown to be DPA > 4NDPA > 2NDPA.

In general, the smokeless powders are distinguishable by the presence or absence of certain compounds. For example, the detection of EC in IMR 4831 can be used to show the difference between this powder and IMR 4350. The EICs for these powder samples are shown in Figures 4.2 and 4.3. While both powders contain the compounds DPA, NsDPA, 4NDPA, 2NDPA, MC, and DBP, the detection of EC in IMR 4831 illustrates the advantage of the HPLC-ESIMS analysis method to differentiate smokeless powders.
Table 4.1. Retention factor ($k'$), intra- and inter-assay precision (percent relative standard deviation, %RSD) of EIC peak areas using the HPLC-ESIMS method for the determination of smokeless powder additives for the standard sample of 1.0 µg/mL ($n \geq 3$). Gradient elution of 50-95% methanol in 25 min. and Pinnacle octyl column.

<table>
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<tr>
<th>Compound</th>
<th>$m/z$</th>
<th>$[\text{M+H}]^+$</th>
<th>$k'$ (within day)</th>
<th>$k'$ (day-to-day)</th>
<th>$%\text{RSD} (n \geq 3)$</th>
<th>$%\text{RSD} (n \geq 3)$</th>
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<td>5.1</td>
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<td>0.5</td>
<td>7.5</td>
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<td>0.4</td>
<td>5.2</td>
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<td>0.4</td>
<td>3.2</td>
<td>6.0</td>
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<tr>
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<td>0.3</td>
<td>6.1</td>
<td>5.3</td>
<td></td>
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<tr>
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<td>2.3</td>
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<td>Ethyl centralite</td>
<td>269</td>
<td>16.1</td>
<td>0.3</td>
<td>3.1</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Dibutylphthalate</td>
<td>279</td>
<td>16.8</td>
<td>0.3</td>
<td>3.8</td>
<td>7.1</td>
<td></td>
</tr>
</tbody>
</table>

Other powders with similar compositions required more extensive analysis. For example, DPA, NsDPA, 4NDPA, 2NDPA, EC, and DBP were detected in H414, H335,
and IMR 4064. Examination of the percent composition in Table 4.2 shows that these powders can easily be distinguished by different concentrations of specific components. Similar levels of DPA were detected in all three powders but H414 and H335 contain a significantly larger percentage of DBP. H414 and H335 can be distinguished by the difference in the percent $N$sDPA and EC detected, for which $t$-tests at the 95% confidence level result in the probability of having equal concentration as 0.01 and 0.02%, respectively. Another example of the applicability of the HPLC-ESIMS method was the detection of 4-4′-dinitrodiphenylamine in IMR 4895. The presence of this dinitro isomer indicates that the powder may have been aged more than the others prior to analysis.

Figure 4.4 shows the extracted ion chromatograms for IMR 4895, in which DPA, $N$sDPA, 4NDPA, 2NDPA, EC, 4-4′-DNDPA, and DBP were detected. The mass spectrum of 4-4′-DNDPA shown in Figure 4.5 illustrates the detection of the protonated molecule of 4-4′-DNDPA, $m/z$ 260, and its sodium adduct, $m/z$ 282. The detection of the 2NDPA isomer in RD900 and 4-4′-DNDPA in IMR 4895 demonstrates the effectiveness of the HPLC-ESIMS method in determining minor compositional differences in smokeless powders.
Table 4.2. Percent composition (%) and standard deviation (s.d., \( n \geq 3 \)) of unburned smokeless powder samples determined using methylene chloride extraction and analysis by the HPLC-ESIMS method with the Pinnacle octyl column and methanol gradient.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( m/z )</th>
<th>AL8 % (s.d.)</th>
<th>H322 % (s.d.)</th>
<th>H414 % (s.d.)</th>
<th>H335 % (s.d.)</th>
<th>2400 % (s.d.)</th>
<th>RD900 % (s.d.)</th>
<th>N130 % (s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethylphthalate</td>
<td>223</td>
<td>0.4 (0.03)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-4’-dinitrodiphenylamine</td>
<td>260</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N )-nitrosodiphenylamine</td>
<td>199</td>
<td>2.0 (0.3)</td>
<td>1.0 (0.08)</td>
<td>0.8 (0.1)</td>
<td>1.3 (0.1)</td>
<td>1.0 (0.3)</td>
<td>0.1 (0.06)</td>
<td>0.4 (0.06)</td>
</tr>
<tr>
<td>Methyl centralite</td>
<td>241</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.7 (0.06)</td>
<td></td>
</tr>
<tr>
<td>4-nitrodiphenylamine</td>
<td>215</td>
<td>0.7 (0.1)</td>
<td>0.2 (0.02)</td>
<td>0.1 (0.01)</td>
<td>0.2 (0.02)</td>
<td>0.9 (0.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>170</td>
<td>2.0 (0.3)</td>
<td>5.2 (0.4)</td>
<td>2.9 (0.3)</td>
<td>3.9 (0.4)</td>
<td>3.5 (0.5)</td>
<td>2.2 (0.8)</td>
<td>3.4 (0.08)</td>
</tr>
<tr>
<td>2-nitrodiphenylamine</td>
<td>215</td>
<td>0.8 (0.1)</td>
<td>0.2 (0.02)</td>
<td>0.4 (0.06)</td>
<td>0.5 (0.1)</td>
<td>0.7 (0.2)</td>
<td>0.1 (0.02)</td>
<td></td>
</tr>
<tr>
<td>Ehtyl centralite</td>
<td>269</td>
<td>0.2 (0.03)</td>
<td>0.3 (0.03)</td>
<td>0.2 (0.04)</td>
<td>0.5 (0.04)</td>
<td>4.5 (0.8)</td>
<td>5.0 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Dibutylphthalate</td>
<td>279</td>
<td>0.4 (0.03)</td>
<td>2.0 (0.1)</td>
<td>2.3 (0.4)</td>
<td>0.5 (0.02)</td>
<td>0.03 (0.01)</td>
<td></td>
<td></td>
</tr>
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</table>
### Table 4.2 (continued).

<table>
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<tr>
<th>Compound</th>
<th>m/z</th>
<th>%</th>
<th>(s.d.)</th>
<th>%</th>
<th>(s.d.)</th>
<th>%</th>
<th>(s.d.)</th>
<th>%</th>
<th>(s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethylphthalate</td>
<td>223</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-4’-dinitrophenylamine</td>
<td>260</td>
<td>0.05</td>
<td>(0.01)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-nitrosodiphenylamine</td>
<td>199</td>
<td>0.8</td>
<td>(0.04)</td>
<td>1.0</td>
<td>(0.2)</td>
<td>1.6</td>
<td>(0.2)</td>
<td>1.4</td>
<td>(0.3)</td>
</tr>
<tr>
<td>Methyl centralite</td>
<td>241</td>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>(0.01)</td>
<td>0.1</td>
<td>(0.03)</td>
<td></td>
</tr>
<tr>
<td>4-nitrodiphenylamine</td>
<td>215</td>
<td>0.4</td>
<td>(0.04)</td>
<td>0.2</td>
<td>(0.04)</td>
<td>0.2</td>
<td>(0.1)</td>
<td>0.2</td>
<td>(0.05)</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>170</td>
<td>0.6</td>
<td>(0.05)</td>
<td>5.7</td>
<td>(1.3)</td>
<td>3.7</td>
<td>(0.6)</td>
<td>3.3</td>
<td>(0.8)</td>
</tr>
<tr>
<td>2-nitrodiphenylamine</td>
<td>215</td>
<td>0.5</td>
<td>(0.05)</td>
<td>0.3</td>
<td>(0.07)</td>
<td>0.5</td>
<td>(0.1)</td>
<td>0.4</td>
<td>(0.1)</td>
</tr>
<tr>
<td>Ehtyl centralite</td>
<td>269</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Dibutylphthalate</td>
<td>279</td>
<td>0.04</td>
<td>(0.004)</td>
<td>0.06</td>
<td>(0.01)</td>
<td>0.03</td>
<td>(0.01)</td>
<td>0.4</td>
<td>(0.06)</td>
</tr>
</tbody>
</table>
Figure 4.1. Extracted ion chromatograms of Red Dot 900 smokeless powder showing 2-nitrodiphenylamine, $m/z$ 215, without the 4-nitrodiphenylamine isomer. HPLC-ESIMS method using gradient elution, 50-95% MeOH in 25min. and the Pinnacle octyl column.
Figure 4.2. Extracted ion chromatograms of IMR 4350 smokeless powder illustrating the presence of diphenylamine, \( N \)-nitrosodiphenylamine, 4- and 2-nitrodiphenylamine isomers, methyl centralite, ethyl centralite, and dibutylphthalate. Conditions as listed in Table 4.1 and Figure 4.1.
Figure 4.3. Extracted ion chromatograms of IMR 4831 smokeless powder, in which diphenylamine, N-nitrosodiphenylamine, 4- and 2-nitrodiphenylamine isomers, methyl centralite and dibutylphthalate were detected. Conditions as listed in Table 4.1 and Figure 4.1.
The results from this study were used to identify distinct compositions among smokeless powders. The identification of the components with the gradient reversed-phase HPLC-ESIMS method produces a profile of the different additives in smokeless powders. This profile has been used for the comparison of different commercially available powders. Previous studies on smokeless powder additives from GSR using ESIMS/MS quantified MC as well as DPA, NsDPA, and 4NDPA but only determined these additives in a limited number of smokeless powders.\textsuperscript{54,55} In this study, the gradient reversed-phase HPLC-ESIMS method was employed to characterize several smokeless powders by simultaneously quantifying their organic additives including diphenylamines, centralites, and phthalates.

Despite demonstrating the comprehensive analysis of smokeless powder additives in this study, the optimization of the solution parameters focused on the HPLC separation but was limited with respect to the ESIMS detection of the additives as protonated molecules. The ionization of neutral analytes in positive ion ESIMS depends on a combination of factors. Using reversed-phase HPLC, gradient elution is often applied without taking into account changes in the solution properties and their effects on the ESIMS intensity. During initial development of the gradient HPLC-ESIMS method, 1 mM ammonium acetate was used in the aqueous fraction of the mobile phase. Consequently, the electrolyte concentration decreased along the elution profile as the percent methanol increased. The influence of decreasing ammonium acetate concentrations on ionization was not apparent during the initial stages of method
development. Therefore, the ionization efficiency of the smokeless powder additives was evaluated as a function of the methanol gradient.

**E. Positive Ion ESIMS Ionization Efficiency**

The solution parameters associated with gradient elution reversed-phase HPLC were characterized by evaluating the ESIMS response of selected smokeless powder additives. Using DLI, the positive ion ESIMS responses were determined for three general classes of powder constituents: dialkylphthalate acid esters, \( N,N'-\)dialkyl-\( N,N'' \)-diphenyl urea based stabilizers, in addition to nitroso-, nitro-, and dinitro- derivatives of diphenylamine. The relative ESIMS intensities of the powder components were investigated as a function of three solution parameters: ammonium acetate concentration, pH, and percent methanol. The results of the characterization experiments were applied to the development of a second gradient reversed-phase HPLC-ESIMS method. The analysis of the selected smokeless powder additives was performed using the new method to demonstrate increased sensitivity of the protonated molecules.
Figure 4.4. Extracted ion chromatograms of IMR 4895 smokeless powder, in which diphenylamine, N-nitrosodiphenylamine, 4- and 2-nitrodiphenylamine isomers, 4,4′-dinitrodiphenylamine and dibutylphthalate were detected. Conditions as listed in Table 1 and Figure 1.
Figure 4.5. Mass spectrum of 4,4'-dinitrodiphenylamine. Collected during chromatographic run of IMR 4895, conditions as listed in Table 4.1 and Figure 4.1.
1. Evaluation of the Electrospray Ionization Mass Spectrometry Response

In order to evaluate the HPLC-ESIMS method, the ESIMS responses of the selected smokeless powder additives were determined as a function of the methanol gradient by DLI with different levels of methanol and 1 mM aqueous ammonium acetate. The decreasing ammonium acetate concentration had a significant effect on the response of the selected smokeless powder components as shown in Figure 4.6. To optimize the solution parameters, the ESIMS responses of the selected analytes were evaluated as a function of three solution parameters including ammonium acetate concentration, aqueous solution pH, and methanol level. The influence of the ammonium acetate concentration was evaluated at different methanol levels with both decreasing and constant electrolyte concentrations to investigate the interaction of these two factors. It should be noted that the goal was to evaluate the ESIMS response as a function of each solution parameter. Thus, these experiments were designed with the sample preparation and run order randomized.

2. Effect of Ammonium Acetate Concentration

During the initial development of the gradient HPLC-ESIMS method with 1 mM ammonium acetate in the aqueous phase, the response of the selected analytes were affected by the methanol gradient. The initial results indicated that the ESIMS intensity was correlated with the ammonium acetate concentration at increasing methanol levels. As shown in Figure 4.6, the signal decreased with decreasing ammonium acetate
concentrations. The relative ESIMS intensities of the compounds in Figure 4.6 were: EC (m/z 269), MC (m/z 241), DBP (m/z 279), DMP (m/z 195), DPA (m/z 170), 4NDPA (m/z 215), NsDPA (m/z 199), and 24DNDPA (m/z 260). The ESIMS response was evaluated with increasing methanol levels and decreasing ammonium acetate concentration. By changing these two solution parameters simultaneously, their influence was not apparent. Therefore, the effects of these two parameters were investigated separately. The ammonium acetate concentration was tested from 0-10 mM. Figure 4.7 shows the ESIMS response of the selected analytes at different ammonium acetate concentrations in 50% methanol. Ammonium acetate was required for efficient ionization as shown by the increased intensity compared to 0 mM and optimal for most compounds at 2 mM. Additionally, at the higher concentrations of ammonium acetate, the intensity decreases due to ionization suppression. Exceptions are shown in Figure 4.7 for 4NDPA and 24DNDPA. No significant difference was observed between the response of 4NDPA at 5 and 10 mM ammonium acetate. As shown in Figures 4.6 and 4.7, the ammonium acetate concentration affected the intensity of 24DNDPA differently compared to the other analytes. From 1.5 to 0.5 mM, the ammonium acetate concentration shown in Figure 4.6 did not have a significant effect on the ESIMS response of 24DNDPA. Figure 4.7 shows that the intensity of 24DNDPA at 5 mM was superior to the other ammonium acetate concentrations. The effect of the ammonium acetate concentration on the mechanism of protonation of 24DNDPA can be explained by the equilibrium partitioning model. The formation of an intramolecular hydrogen bond
between the oxygen of the 2-nitro group and the amine hydrogen may cause an apparent decrease in the polarity of 24DNDPA in solution and within the electrosprayed droplet.

3. Effect of Solution pH

An alternative justification of the ESIMS response decrease shown in Figure 4.6 is that the signal decreases with the level of water or available protons in the infused sample. Thus, the effect of the aqueous solution pH was investigated in 50% methanol/ 2 mM aqueous ammonium acetate. As shown in Figure 4.8, the pH of the aqueous solution did not have a significant effect on the ESIMS intensity of the selected analytes.
Figure 4.6. ESIMS response of smokeless powder additives as a function of the percent methanol (lower scale) and decreasing ammonium acetate concentrations (upper scale).
Figure 4.7. ESIMS response of smokeless powder additives at different ammonium acetate concentrations in 50% methanol/water.
It should be noted that the pH values shown in Figure 4.8 were determined experimentally for the aqueous solution without correction for dilution in methanol. The pH of the original 2 mM aqueous ammonium acetate was measured at pH 6.7. Because the pH of the aqueous electrolyte solution was adjusted by the addition of a 1% (v/v) acetic acid containing 2 mM ammonium acetate, the concentration of available protons increased at pH 4.7 and 5.3. Alternatively, with the addition of ammonium hydroxide, the concentration of available protons decreased at pH 8.3. Thus, the negligible effect of pH on the ESIMS intensity illustrates that the protonation of the selected analytes are more dependent upon the gas-phase proton affinity than the level of available protons in solution, which has been described previously for other weakly basic compounds.\textsuperscript{49,77-79}

The responses of 4NDPA and 24DNDPA actually increased with increasing pH. This effect demonstrates that the mechanism of protonation for these compounds may be different than the other additives. Although the specific factor is not known, it is proposed that the gas-phase proton affinities of the nitro-substituted constituents are energetically favored over ammonia.\textsuperscript{79}
Figure 4.8. ESIMS intensity of the selected analytes illustrating the effect of the aqueous solution pH in 50% methanol/ 2 mM aqueous ammonium acetate.
4. Effect of Percent Methanol

As shown in Figure 4.6, the ESIMS intensity decreased with increasing methanol levels and was correlated with the ammonium acetate concentration in the aqueous fraction of the mobile phase. Thus, the influence of the two factors was confounded and the effect of the percent methanol alone was not obvious. While maintaining a constant ammonium acetate concentration of 2 mM, the effect of the percent methanol was investigated. Figure 4.9 shows the ESIMS intensity as a function of the methanol level with the concentration of ammonium acetate held constant at 2 mM. The ESIMS signal generally increases with the percent methanol. However, the intensities of NsDPA, 4NDPA, and 24DNDPA do not significantly increase with increasing percent methanol. Previous reports regarding the ESIMS response as a function of organic solvent levels attributed variations in the intensity of ionic analytes to the surface activity and ion mobility of the analyte within the electrosprayed droplet.\textsuperscript{80,81} The intensity increase shown in Figure 4.9 for most compounds was not observed with decreasing ammonium acetate concentrations in Figure 4.6. It is proposed that the electrolyte level was the prominent factor in both cases of increasing percent methanol. In Figure 4.6, the ammonium acetate concentration was below the optimal level, which reduced droplet stability and analyte transfer into the gas phase. Conversely, the intensity increase shown in Figure 4.9 was credited to the reduced surface tension of the solution whereas the ammonium acetate concentration of 2 mM maintained droplet stability. Because a mixture of several analytes was infused, no specific property was found to be responsible
for the ESIMS response of 

N{sDPA, 2DNDPA, and 24DNDPA as a function of the percent methanol with 2 mM ammonium acetate. In fact, several factors may be responsible for the ESIMS response as a function of the solution parameters investigated in the present study.

F. Optimized Conditions for HPLC-ESIMS

The ESIMS responses of smokeless powder additives have been characterized as a function of solution parameters related to the gradient elution profile in the present study. The results demonstrate that the ESIMS intensities of the smokeless powder additives were dependent upon the ammonium acetate concentration with varying and constant levels of methanol, not affected by pH, and increased with the percent methanol with the electrolyte concentration held constant. The effects of these solution parameters were tested by comparing the results of the HPLC-ESIMS method validation using two different mobile phase compositions.

The analysis of standard mixtures of the selected analytes with concentrations between 0.05-20 µg/mL was used for method validation of the new mobile phase, which consisted of methanol and water with 2 mM ammonium acetate in both organic and aqueous portions with no pH control. Linear regression was performed using the EIC peak areas for each smokeless powder additive to establish the method sensitivities. The slopes of the regression lines using the two different mobile phase compositions are compared in Figure 4.10. The method sensitivity using 2 mM total ammonium acetate concentration in the mobile phase was superior for the selected analytes, except DPA
The inset in Figure 4.10 illustrates the increased response using 2 mM total ammonium acetate concentration for the extracted ion chromatograms for 24DNDPA at 10 µg/mL.

G. Determination of Smokeless Powder Components

The new HPLC-ESIMS method was validated using linear regression data for the EIC peak areas of each component over the concentration range of 0.01 to 25 µg/mL. The retention factor ($k'$), detection limit and accuracy of the smokeless powder additives are given in Table 4.3. Compared to the retention factors in Table 4.1, the $k'$ values using new HPLC-ESIMS method were similar for early eluting peaks. However, the retention factors of the compounds eluting after 4NDPA ($t_r = 13$ min.) increased.

The detection limits given in Table 4.3 were calculated from the linear calibration data using $S/N = 3$. As shown in Figure 4.10, the sensitivity of the method improved for the selected powder additives by changing the ammonium acetate concentration, which was verified using two-way ANOVA ($p = 0.02$). The accuracy, defined as the absolute error was calculated by determining the average deviation of the experimental concentration from the expected concentration using the calibration data over the range 0.1-25 µg/mL. With the exception of 4sNDPA, the accuracy of the calibration data for the smokeless powder additives was within ± 0.1 µg/mL as shown in Table 4.3.

The results obtained using the new HPLC-ESIMS method can be correlated to the inferences from the ionization efficiency investigations and previous studies that
demonstrated the increased intensity for more hydrophobic molecules.\textsuperscript{53} While the hydrophobic compounds were retained longer on the column, the droplet stability was enhanced as a result of increasing the ammonium acetate concentration in the mobile phase.
Figure 4.9. The ESIMS intensity of smokeless powder additives as a function of percent methanol with the concentration of ammonium acetate held constant at 2 mM.
Figure 4.10. Comparison of method sensitivities (slope of the regression line) using 1 mM ammonium acetate in the aqueous fraction and 2 mM total ammonium acetate concentration in both organic and aqueous portions of the mobile phase. The inset illustrates the increased ESIMS response using 2 mM total ammonium acetate concentration for the extracted ion chromatograms for 24DNDPA.
Table 4.3. Retention factor ($k'$), detection limit (±95% confidence interval) and accuracy (absolute error) of smokeless powder additives determined by the HPLC-ESIMS method with the Pinnacle octyl column and methanol gradient with 2 mM in the mobile phase.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>$m/z$</th>
<th>$k'$</th>
<th>[M+H]$^+$ Detection Limit</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethylphthalate</td>
<td>DMP</td>
<td>195</td>
<td>7.7</td>
<td>1.0 ± 0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Diethylphthalate</td>
<td>DEP</td>
<td>223</td>
<td>10.7</td>
<td>1.3 ± 0.3</td>
<td>0.06</td>
</tr>
<tr>
<td>4-Nitrosodiphenylamine</td>
<td>4sNDPA</td>
<td>199</td>
<td>10.9</td>
<td>0.5 ± 0.2</td>
<td>-0.2</td>
</tr>
<tr>
<td>4-4'-Dinitrodiphenylamine</td>
<td>44DNDPA</td>
<td>260</td>
<td>12.1</td>
<td>1.5 ± 0.2</td>
<td>-0.03</td>
</tr>
<tr>
<td>N'-Nitrosodiphenylamine</td>
<td>NsDPA</td>
<td>199</td>
<td>12.5</td>
<td>1.2 ± 0.5</td>
<td>-0.06</td>
</tr>
<tr>
<td>2-4'-Dinitrodiphenylamine</td>
<td>24DNDPA</td>
<td>260</td>
<td>12.8</td>
<td>0.54 ± 0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Methyl Centralite</td>
<td>MC</td>
<td>241</td>
<td>12.9</td>
<td>0.5 ± 0.1</td>
<td>0.04</td>
</tr>
<tr>
<td>4-Nitrodiphenylamine</td>
<td>4NDPA</td>
<td>215</td>
<td>13.3</td>
<td>0.5 ± 0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>DPA</td>
<td>170</td>
<td>14.0</td>
<td>1.8 ± 0.3</td>
<td>0.04</td>
</tr>
<tr>
<td>2-Nitrodiphenylamine</td>
<td>2NDPA</td>
<td>215</td>
<td>16.0</td>
<td>1.7 ± 0.1</td>
<td>0.09</td>
</tr>
<tr>
<td>Ethyl Centralite</td>
<td>EC</td>
<td>269</td>
<td>16.7</td>
<td>0.9 ± 0.1</td>
<td>-0.09</td>
</tr>
<tr>
<td>Dibutylphthalate</td>
<td>DBP</td>
<td>279</td>
<td>19.2</td>
<td>0.40 ± 0.04</td>
<td>0.08</td>
</tr>
</tbody>
</table>

1. Analysis of Unburned Smokeless Powders

The quantitative analysis of several unburned smokeless powders was performed using the new HPLC-ESIMS method with constant ammonium acetate concentration of 2 mM throughout the linear gradient. In the previous experiments, only unburned powders
were analyzed to differentiate various powders from different sources. In the present study, unburned powders from the same manufacturer but different lots were analyzed. The analysis of pipe bomb residue using these powders from different lots was also included in this study to demonstrate the utility of the revised HPLC-ESIMS method.

The percent composition of the additives in twelve unburned smokeless powders is given in Table 4.4. With the exception of a single sample of RD900, the smokeless powders comprised four groups from different manufacturers with at least two different lots of each powder. Four different lots of Hi-Skor 700X (H-S 700-X), three lots of IMR 4895, and two lots each of Winchester 296 (WIN 296) and H380 were analyzed as unburned samples and as residue from the pipe bombs. The differentiation of the unburned smokeless powders, as described previously, can be accomplished by comparing the composition of each powder. For example, while 4NDPA and DBP were detected in all the powders, the two lots of Hodgden 380 (H380) were the only powders in which 44DNDPA was detected.
Table 4.4. Composition (%) of unburned smokeless powder samples determined using methylene chloride extraction and analysis by the HPLC-ESIMS method with the Pinnacle octyl column and methanol gradient with 2 mM in the mobile phase.

<table>
<thead>
<tr>
<th>Powder</th>
<th>RD900</th>
<th>WIN 296</th>
<th>WIN 296</th>
<th>H380</th>
<th>H380</th>
<th>H-S 700-X</th>
<th>H-S 700-X</th>
<th>H-S 700-X</th>
<th>H-S 700-X</th>
<th>IMR 4895</th>
<th>IMR 4895</th>
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<td></td>
<td>869</td>
<td>818</td>
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<tr>
<td>Compound</td>
<td>m/z</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>4,4’-dinitrodiphenylamine</td>
<td>260</td>
<td>5</td>
<td>5</td>
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<tr>
<td>N-nitrosodiphenylamine</td>
<td>199</td>
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<td>0.02</td>
<td></td>
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<tr>
<td>Methyl centralite</td>
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<td></td>
<td></td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.006</td>
<td>0.009</td>
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<tr>
<td>4-nitrodiphenylamine</td>
<td>215</td>
<td>0.002</td>
<td>0.09</td>
<td>0.09</td>
<td>0.1</td>
<td>0.09</td>
<td>0.002</td>
<td>0.003</td>
<td>0.005</td>
<td>0.07</td>
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<td>4</td>
<td>5</td>
<td>4</td>
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<tr>
<td>2-nitrodiphenylamine</td>
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<td>0.002</td>
<td>0.1</td>
<td>0.08</td>
<td>0.09</td>
<td>0.09</td>
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<tr>
<td>Ethyl centralite</td>
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<td>0.08</td>
<td>0.05</td>
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<td>1</td>
<td>4</td>
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<tr>
<td>Dibutylphthalate</td>
<td>279</td>
<td>0.01</td>
<td>2</td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.002</td>
<td>0.004</td>
<td>0.006</td>
<td>0.002</td>
<td>0.02</td>
<td>0.002</td>
</tr>
</tbody>
</table>
2. Analysis of Pipe Bomb Residue

The estimated percent composition of the additives in the pipe bomb residue samples of 12 smokeless powders is given in Table 4.5. The concentration of the additives was estimated because the amount of smokeless powder in the residue was unknown. Because the pipe bomb residues were extracted with larger amounts of methylene chloride and a greater volume reduction, the percent composition calculation yielded similar results using a divisor of 25 rather than ~5 used with the unburned powders. Although different extraction volumes were used, the qualitative analysis of the chromatographic results can be used for sample comparison. Like the unburned samples, all the pipe bomb residue samples show the presence of 4NDPA and DBP as shown in Table 4.5. Additionally, all the pipe bomb samples contained 2NDPA. The qualitative distinction of the different powders and within the same powder but different lots is shown by the presence or absence of DPA and its isomers. For example, three of the powders in the four lots of H-S700-X do not contain DPA. In addition, two of the three contain 24DNDPA. Similarly, the two lots of WIN 296 can be differentiated by the presence of 24DNDPA in lot D901. Figure 4.11 shows the extracted ion chromatograms of the H-S 700-X lot 818 pipe bomb sample and unburned powder extract. While Figure 4.11 illustrates that both samples contain NsDPA, 4NDPA, and MC, it also demonstrates the sensitivity of the novel HPLC-ESIMS method. Tables 4.4 and 4.5 as well as Figure 4.11 show that the amount of NsDPA decreased but the level of 4NDPA remained the same in lot 818 of H-S 700-X. The decrease in the concentration of NsDPA is a likely
result from either the oxidation of NsDPA to 4NDPA or a greater level of decomposition of NsDPA in the pipe bomb sample. These conclusions are consistent with the nitration and nitrosation reactions of DPA under conditions representative of smokeless powder storage.⁴,⁵ Although the reactions that occur during the deflagration of the powder in the pipe bombs are unknown, the decrease in the concentration of NsDPA as shown in Figure 4.11 could be elucidated by using this method under carefully controlled conditions. Because the HPLC-ESIMS method allows for the quantitative comparison of minor compositional changes in smokeless powder additives, this novel method would be useful for revealing reaction products as a result of deflagration. For example, Figure 4.12 shows the extracted ion chromatograms for the comparison of D61A lot of Winchester 296 pipe bomb sample and unburned powder extract. While the pipe bomb residue contained 24DNDPA and 44DNDPA, the unburned powder did not show the presence of these isomers.
Table 4.5. Composition (%) of pipe bomb residues determined using methylene chloride extraction and analysis by the HPLC-ESIMS method with the Pinnacle octyl column and methanol gradient with 2 mM in the mobile phase. Unburned smokeless powder same as in Table 4.4.

<table>
<thead>
<tr>
<th>Powder</th>
<th>Lot No.</th>
<th>Compound</th>
<th>m/z</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
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<td></td>
<td></td>
<td>RD900</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>D61A</td>
<td>WIN 296</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>B5A</td>
<td>WIN 296</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>869</td>
<td>H-S 700-X</td>
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<tr>
<td></td>
<td>926</td>
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</tr>
</tbody>
</table>

- **44DNDPA**: 260, 0.0005, 0.4, 0.1, 0.02, 0.03, 0.1
- **NsDPA**: 199, 0.05, 1, 0.7, 0.5, 0.8, 0.003, 0.02, 0.79, 0.72, 0.69
- **24DNDPA**: 260, 0.001, 0.08, 0.03, 0.06, 0.05, 0.02, 0.006, 0.03, 0.04
- **MC**: 241
- **4NDPA**: 215, 0.005, 0.09, 0.01, 0.01, 0.001, 0.003, 0.002, 0.0001, 0.02, 0.01, 0.01
- **DPA**: 170, 0.01, 0.2, 0.2, 0.3, 0.002, 0.4, 0.5, 0.2
- **2NDPA**: 215, 0.001, 0.004, 0.002, 0.002, 0.004, 0.002, 0.02, 0.004, 0.068, 0.008, 0.006, 0.005
- **EC**: 269, 0.02, 0.03, 0.03, 0.1, 0.2, 0.2, 0.2, 0.2
- **DBP**: 279, 0.009, 0.2, 0.1, 0.02, 0.03, 0.0002, 0.0005, 0.0002, 0.0003, 0.004, 0.06, 0.001
Figure 4.11. Comparison of H-S 700-X lot 818 pipe bomb sample and unburned powder extract using gradient HPLC-ESIMS with 2 mM total ammonium acetate concentration in the mobile phase.
Figure 4.12. Comparison of D61A lot of Winchester 296 pipe bomb sample and unburned powder extract using gradient HPLC-ESIMS with 2 mM total ammonium acetate concentration in the mobile phase.
H. Conclusions

The goal in the forensic analysis of smokeless powders is to distinguish individual samples by characterizing and identifying the constituents. An HPLC-ESIMS method was developed for the simultaneous determination of the common organic additives in smokeless powders. The ESIMS was optimized for the detection of protonated molecules in the full scan positive ion mode. The separation of compounds with varying polarity was accomplished by using the octyl column with the methanol gradient. For example, $N$-nitroso-, 4-nitroso-, 4-nitro- and 2-nitro- derivatives of DPA, which are reaction products of nitrous and nitric acid with DPA, were separated and quantified. The gradient reversed-phase HPLC-ESIMS method was used to differentiate several smokeless powders by their additive profile. Because this method can be used to determine quantitative results for the selected components, it should prove useful in the analysis of compositional variation and smokeless powder degradation.
V. Chapter 5. Survey of Environmental Background Interferences Affecting High Explosives Residue Analysis

A. Introduction

The goal of this project was to develop and validate instrumental methods for the forensic analysis of trace organic explosives in public places. The portion of the project included in this research involved GC methods development for the separation and selective detection of six high explosives. The separation of EGDN, NG, TNT, RDX, PETN, and HMX were performed using two GC systems. The retention characteristics of the six explosives were evaluated using the GC/ECD with a large bore column and high flow rate. The recoveries of the explosives were evaluated by extracting standards from dry cotton swabs with acetone followed by analysis using GC/ECD. The separation and identification of the explosives EGDN, NG, TNT, PETN, and RDX were performed by GC-MS with methane NCI to validate the confirmation method.

B. Method Development

1. GC/ECD

The separation and quantification of the six explosives EGDN, NG, TNT, RDX, PETN, and HMX were evaluated using standard mixtures over the concentration range 1-1000 ng/mL. Several methods were evaluated for the separation of the six explosives prior to determining the optimal parameters. The recoveries of the explosives were
evaluated by extracting standard mixtures from dry cotton swabs with acetone followed by analysis using GC/ECD.

The initial parameters of the GC/ECD method were taken from previous studies on explosives analysis. [Miller, 2002 #221] While the analysis of several explosives have been evaluated using GC methods, the detection of HMX has only recently been reported. In this study, the important parameters for the analysis of the six explosives were the injection temperature, carrier flow rate and temperature program. By using a large bore column and high flow rate the degradation of the thermally labile compounds was minimized. The optimal parameters were determined by evaluating the retention factors, peak shapes and the reproducibility of the responses. An injection temperature of 180 °C, carrier flow rate of 35 mL/min., and temperature program of 50 °C for 1 min. to 150 °C at 20 °C/min., to 250 °C at 40 °C/min. were established as the optimal parameters to provide a rapid screening method. A typical chromatogram of a 25 ng/mL standard mixture of the explosives (250 ng/mL HMX) is shown in Figure 5.1. The retention factors, limits of detection, and percent recoveries are listed in Table 5.1. The limits of detection were determined from the linear calibration data using $S/N = 3$. While the method was capable of detecting low levels of the energetic compounds using the optimal parameters, the retention factors were maximized in order to screen various types of unknown samples.

Standard mixtures containing 50-5000 ng/mL HMX and 1-100 ng/mL of the other standards were used to examine the method reproducibility. The precision for the retention factors were less than 5% for all calibration standards ($n = 30$ runs). The
The linearity of the calibration data, expressed as the coefficient of determination ($r^2$) was 0.999 or greater. Although the detection limit of HMX was much greater than the other explosives, the values given in Table 5.1 correspond to pg levels on-column using 1 µL injections.

Table 5.1. Retention factor ($k'$), detection limit ($S/N = 3$), and percent recovery of explosives determined by GC/ECD (± 95% confidence interval).

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k'$</th>
<th>Detection Limit (ng/mL)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGDN</td>
<td>14±1</td>
<td>2.0±0.7</td>
<td>87±1</td>
</tr>
<tr>
<td>NG</td>
<td>27±1</td>
<td>4±1</td>
<td>70±2</td>
</tr>
<tr>
<td>TNT</td>
<td>44±2</td>
<td>10±3</td>
<td>76±5</td>
</tr>
<tr>
<td>PETN</td>
<td>49±2</td>
<td>2.0±0.1</td>
<td>83±1</td>
</tr>
<tr>
<td>RDX</td>
<td>52±2</td>
<td>9±3</td>
<td>52±5</td>
</tr>
<tr>
<td>HMX</td>
<td>81±4</td>
<td>160±50</td>
<td>15±8</td>
</tr>
</tbody>
</table>

**a. Evaluation of High Explosives Recovery**

The recovery of the six explosives was evaluated by extracting standard mixtures from cotton swabs with acetone. The recovery was defined as the concentration of the explosive in the acetone extract as determined by external calibration. The preliminary determination of recovery in Table 5.1 showed that there was limited extraction efficiency using this method. The use of internal standards is not practical due to the large number of samples to be analyzed during the lifetime of the project. In addition, the
analytes have considerably different molecular structures, which would require the use of different internal standards for each class of explosive. The extraction efficiency was reassessed by using calibration samples throughout the extraction procedure. Using the calibration standards all the way through the extraction, all explosives were extracted at 100% within the experimental error.

The sample preparation procedure was also evaluated using a volume reduction step to preconcentrate the explosives by evaporating excess solvent in the samples with nitrogen gas. The percent recovery was evaluated for spiked samples from 0.5 to 12 ng on different cotton swabs. The concentrations of the injected samples were based upon estimated volumes. The percent recovery was defined by comparing the estimated concentration of the spiked sample to the concentration determined experimentally.
Figure 5.1. GC/ECD chromatogram of a standard mixture of the six explosives. The mixture contained 25 μg/mL of EGDN, NG, TNT, RDX, PETN, and 250 μg/mL HMX.
The concentration range evaluated in these experiments approached the detection limits of the explosives using the GC/ECD method. In addition, considerable variation was introduced by estimating the concentration of the injected sample from the volume reduction step. The percent recovery for spiked samples is shown in Figure 5.2, in which the error bars represent the 95% confidence interval for pooled standard deviations across the concentration range. Although increased variation was anticipated by using the estimated volumes and low concentrations, the results indicate that significant amounts of the explosives were recovered. The percent recoveries were 100% for all compounds. Notable deviations are shown for NG and RDX, in which the recoveries were significantly greater than 100%. It was expected that a trend of reduced response as a function of volatility would result from the volume reduction stage of sample preparation. However, no such trend was observed. For example, the responses of EGDN and NG were similar to the other less volatile explosives such as PETN.
Figure 5.2. Percent recovery for spiked samples from 0.5 to 12 ng (HMX 10 to 200 ng) determined by GC/ECD, error bars indicate the 95% confidence interval from a pooled standard deviation.
2. GC-MS

The separation and identification of the explosives EGDN, NG, TNT, PETN, and RDX was performed by GC-MS with NCI using methane reagent gas. Two different columns were used during the development of the GC-MS method. The Finnigan GCQ GC-MS was initially equipped with a Chrompak CP8MS column (DB5 type, 12 m, 0.25 mm, 0.25 µm film thickness). Mixtures of explosives standards were used to evaluate the GC-MS response by linear calibration. The limits of detection determined using \( S/N = 3 \) with this narrow column are given in Table 5.2.

The GC-MS was also equipped with a Restek Rtx5MS (DB5 type, 15 m, 0.53 mm, 1.5 µm film thickness) column. Using this large bore column, the effluent was split in the GC oven by sliding the analytical column over a 0.1 mm fused silica transfer line, thus venting part of the flow into the oven. [Miller, 2002 #221] The split technique allows for high flow rates to be used without creating high pressure in the MS while minimizing thermal degradation of certain explosives in the GC oven. The optimum length of the transfer line inside the GC oven was determined by evaluating the peak shape and intensity for a 50 µg/mL standard mixture of the energetic compounds. The transfer line length did not affect the intensity or the retention time from a ~10-40 mm length inside the oven. However, peak tailing was noticeable for PETN and RDX at a total length of 50 mm. The transfer line was used with the large bore column at a length of 10 mm to evaluate the linearity of the MS response. The limits of detection from the GC-MS analyses of standard explosives mixtures from 10-100 µg/mL using the two columns are
listed in Table 5.2. Although the Rtx5MS column was split to a transfer line in the GC oven, the limits of detection were lower using the large bore column as shown in Table 5.2.

Table 5.2. Detection limits (μg/mL) determined by GCMS using two different columns. The column dimensions were Chrompak CP8MS (12 m length, 0.25 mm inner diameter, 0.25 μm film thickness) and Rtx5MS (15 m length, 0.53 mm inner diameter, 1.5 μm film thickness).

<table>
<thead>
<tr>
<th>Compound</th>
<th>CP8MS (0.25mm)</th>
<th>Rtx5MS (0.53mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGDN</td>
<td>11 ± 4</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>NG</td>
<td>19 ± 6</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>TNT</td>
<td>13 ± 5</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>PETN</td>
<td>22 ± 7</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>RDX</td>
<td>18 ± 6</td>
<td>3 ± 2</td>
</tr>
</tbody>
</table>

C. Conclusions

The analysis of six common high explosives was performed using two GC systems with a large bore columns and high flow rates. While these methods have previously been demonstrated for the analysis of certain samples, their use in this study provides a sensitive and fast screening method and a selective mass spectrometry method for unambiguous explosive identification. A rapid and robust sample screening method was developed using GC/ECD. By evaluating the recovery of explosives from cotton
swabs, applying the GC/ECD method allows for the determination of low levels of explosives. The GC-MS method using NCI with methane reagent gas was used for the separation and identification of EGDN, NG, TNT, PETN, and RDX. This selective method takes advantage of the large bore column and splitting the effluent in the GC oven to minimize the thermal degradation of the explosives.
VI. Chapter 6. Analysis of Explosives and Related Compounds by HPLC-ESIMS in the Negative Ion Mode

A. Introduction

HPLC-ESIMS is an alternative to GC methods for the analysis and identification of thermally labile nitrated explosives. Several research studies have been performed to demonstrate the detection of selected explosives in the negative ion mode.\textsuperscript{9-13,38,62,70,72} While these reports have been used for specific applications, the methods have particular shortcomings. For example, the details on instrumental and solvent optimization were inadequate in the report on the ESIMS/MS detection of high explosives.\textsuperscript{11} No discussion was given on the use of electrolytes to promote ionization and there was minimal description of the processes for ion formation. In a later study, nitrate, nitrite, propionate, and chloride adducts of selected nitrate ester explosives were characterized by HPLC-ESIMS and HPLC-APCI-MS.\textsuperscript{59} The HPLC-ESIMS method used post column addition of the anionic additives, which can be difficult to optimize and reproduce. While these studies demonstrate ESIMS detection in the negative ion mode, their practical application in forensic crime laboratories would not be easily implemented as a comprehensive method for a different types of explosives samples.

A successful aspect of the previous studies was the detection of anionic adduct ions.\textsuperscript{10,13,72} However, the theoretical and practical considerations of detecting explosives as anionic adducts have not been thoroughly examined. The goal of the present study is to establish a comprehensive analysis method that provides quantitative and definitive structural information for high explosives. Preliminary studies using negative ion ESIMS
were performed using DLI for the detection of selected high explosives. By adding volatile ammonium salts to the solvent, the formation of explosive-anion adducts were characterized by ranking the intensities of several adducts of high explosives. These studies illustrate that an HPLC-ESIMS method can be used for the separation of explosives with detection as unique anionic adducts. An isocratic reversed-phase HPLC-ESIMS method was developed for the simultaneous analysis of several high explosives. The distinctive method takes advantage of the preferential gas-phase interaction between particular neutral explosives and specific anions.

B. Negative Ion ESIMS Detection of High Explosives

In previous studies, the detection of high explosives adducts with different anions was achieved using negative ion ESIMS.\textsuperscript{10-13} While these reports have been used for particular applications, the methods were limited to specific explosive-anion adduct pairs including EGDN, NG, PETN, and RDX, which were detected individually as adducts with nitrite, chloride, and nitrate. In the present study, the detection of high explosives adducts of chloride and nitrate was examined to compare with the results from previous studies.

Mixtures containing individual explosives and one of the ammonium salts were infused into the mass spectrometer. As anticipated, nitrate adducts were detected in mixtures containing ammonium nitrate and chloride adducts were detected when ammonium chloride was present. At the same time, unexpected results were obtained during the characterization of these adducts. Although only one anion was deliberately
added to the mixture, other explosive-anion adducts were detected. For example, the mass spectrum acquired during the infusion of the mixture containing EGDN and ammonium nitrate showed the presence of the nitrate adduct and the chloride adduct. Figure 6.1 illustrates the detection of the EGDN-chloride \((m/z\ 187)\) adduct that was not expected. While the focus of the experiment was to characterize the EGDN-nitrate \((m/z\ 214)\) adduct, the detection of the chloride adduct was a consequence of the gas-phase interaction of EGDN with chloride that was most likely present in the solution as an impurity.

Because other unanticipated explosive-anion combinations were discovered during these characterization studies, a more comprehensive approach was considered to detect several diagnostic explosives adducts with different anions. The hypothesis is that by intentionally adding specific anionic components, distinctive explosive adducts can be detected. This proposal is based on the preferential gas-phase interaction between the anions and neutral explosives. Further experiments were designed to assess the detection of several explosive-anion adduct species. These studies address the theoretical and practical aspects that are necessary to describe how adducts are formed and the implications of applying negative ion ESIMS methods. The results will be beneficial to forensic laboratories for the detection of explosives by ESIMS.
Figure 6.1. Mass spectrum of EGDN-nitrate, \( m/z \) 214, from a mixture containing, 50% MeOH/50% aqueous 1 mM ammonium nitrate, which also shows presence of the EGDN-chloride adduct, \( m/z \) 187.

1. Relative stability of High Explosives Adducts

The stability of anionic adducts has been attributed to comparable gas-phase basicities of the deprotonated analyte molecule ([M-H]\(^-\)) and the anionic species ([A]\(^-\)).\(^ {67,68} \) The adduct ion has been described as two gas-phase anions that are in equilibrium
with a proton and illustrated as ‘[M-H]•••H+•••[A]’. These previous studies evaluated the ESIMS intensity of neutral compounds without acidic groups with different anionic species using tandem MS studies, which employed CID at a single collision energy. The stability of the adducts ions were described by the relative intensities of their decomposition products, [M-H][ or [A][. This experimental scheme requires that all the compounds form stable deprotonated molecules at the specified collision energy. While the high explosives used in the present studies do not contain acidic groups, the stability of explosive-adduct ions cannot be assessed using tandem MS CID experiments because the deprotonated molecules of all the energetic compounds are not stable. An assessment of the relative adduct stability can be made for individual explosives with different anionic species. The results will be beneficial to forensic laboratories developing negative ion ESIMS methods for the detection of explosives.

The intensities of high explosives adducts of chloride, formate, acetate and nitrate were determined to rank their relative stability. The responses of several high explosives adducts were determined at different concentrations of the ammonium salts from 0 to 5 mM. The variation in the response was not significantly different over the range of concentrations. Figure 6.2 shows the negative ion ESIMS mass spectrum of a high explosive mixture containing in EGDN, NG, TNT, PETN, RDX, and HMX in a 50% aqueous mixture of 0.3 mM ammonium chloride, ammonium formate, and ammonium nitrate. Although the nitrate adducts were the most abundant peaks, the detection of all the adducts of the six explosives with the three anions was accomplished. For example, RDX forms stable adducts with nitrate m/z 284, chloride, m/z 257, formate m/z 267, and
acetate m/z 281. The responses were used to assess the relative adduct stability by determining intensity ratios for each of the explosives. The intensities of the most abundant species were used as the reference to compare the adduct stability of the other anionic adducts. The ratios of the adduct intensity for each explosive are shown in Figure 6.3. While the nitrate adducts of EGDN, NG, PETN, and RDX were the principal species detected for these compounds, the chloride adduct of HMX at m/z 331 was the most abundant adduct for this nitramine. As shown in Figure 6.3, the relative stability of the adducts for EGDN were ranked as nitrate, acetate, formate, and chloride. The relative anionic adduct stability of RDX and PETN were in the order of nitrate, chloride, formate, and acetate. The relative stability of the NG adducts was established as nitrate, chloride, acetate, and formate. The adducts of HMX were ranked as chloride, nitrate, formate, and acetate.
Figure 6.2. Mass spectrum of high explosive mixture containing EGDN, NG, TNT, PETN, RDX, and HMX in 50% MeOH/50% aqueous mixture of 0.3mM ammonium chloride, ammonium formate, and ammonium nitrate.
These results are consistent with previous studies on adduct formation using negative ion ESIMS for the detection of EGDN, NG, PETN and RDX.\textsuperscript{13,38,72} When Although EGDN has previously been detected as chloride, nitrate, nitrite, and propionate adducts, this study is the first report for the detection of the EGDN-formate adduct.\textsuperscript{13}

The negative ion ESIMS detection of EGDN has been demonstrated previously by Yinon and co-workers but the results indicated poor detection limits compared to other high explosive compounds.\textsuperscript{11-13} While previous studies on the detection of EGDN have suggested the use of low operating temperatures and ammonium hydroxide as a mobile phase additive, these parameters were not directly characterized or optimized.\textsuperscript{11,13} Thus, the detection of EGDN-formate adduct was evaluated at different ESIMS operating temperatures and pH levels. In addition to EGDN-formate, $m/z$ 197, the formic acid-formate adduct, $m/z$ 91 was assessed for comparison at the different operating conditions. Two mixtures containing different concentrations of EGDN in 50% IPA/2mM aqueous ammonium formate were used during these characterization studies. The temperature study was performed using 25 \( \mu \)g/mL EGDN and the pH investigations were carried out using 50 \( \mu \)g/mL EGDN at an ESIMS operating temperature of 125 °C. The solution pH was not determined for the solution used at the different operating temperatures. The solution pH was adjusted in the original 2mM aqueous ammonium formate solution (pH 6.2) by adding 10% v/v formic acid or ammonium hydroxide solutions that also contained 2mM ammonium formate.

Figure 6.4 shows the characterization of EGDN-formate, $m/z$ 197 and formic acid-formate adduct, $m/z$ 91 at different temperatures (a) and as a function of solution pH
(b) using negative ion ESIMS. The intensity of the EGDN-formate adduct was constant from 90 to 150 °C but decreased significantly at 200 and 250 °C. Alternatively, the intensity of formic acid-formate adduct was stable from 90 to 200 °C but increased at 250 °C. The effect of the solution pH on the intensities of the EGDN-formate and formic acid-formate adducts was evaluated using two-way ANOVA. The results indicate a significant effect ($p = 0.01$) for both adducts at the different pH values. The increasing solution pH from 7.1 to 8.2 shows a slight increase in the intensity of the EGDN-formate adduct as shown in Figure 6.4b. The low pH levels also had a significant effect on the intensity of the formic acid-formate, $m/z$ 91 adduct. Because the concentration of formic acid in the liquid solution is much higher than free formate below the $pK_a$ (3.75), the increased intensity at pH 3.0 is expected.

C. HPLC-ESIMS

An isocratic reversed-phase HPLC-ESIMS method was developed for the detection of characteristic adducts of high explosives using two different instrumental set-ups to introduce the ammonium salts. Initially, the post column addition of ammonium nitrate and ammonium chloride employed previously by Yinon was used to reproduce the detection of the selected explosives. In the second instrumental design, the volatile ammonium salts were added directly to the mobile phase. The mobile phase consisted of 50% MeOH/50% aqueous mixture containing 0.05 mM ammonium nitrate, and 0.1 mM ammonium salts of formate, acetate and chloride. The analysis of standard
mixtures of high explosives was performed using a C₁₈ column with the ESIMS operated in the negative ion mode.

D. Method Development

The isocratic reversed-phase HPLC-ESIMS method was used for the analysis of standard mixtures. During the development of the separation method, different methanol levels were used to maximize the resolution between the high explosives. In addition, the concentration of the ammonium salts of nitrate, formate, acetate and chloride was important because these species can form clusters over the same m/z range as the high explosives adducts. The ammonium salt concentrations were selected to minimize interferences from the salt clusters. Methanol levels from 30-70% were used during the initial stages of method optimization. While the retention of PETN was dependant on the percent of the organic modifier, the resolution of EGDN, HMX, RDX, NG, and TNT were not significantly different over the range tested. The separation was sufficient at 50% methanol and accomplished in under 10 min.
Figure 6.3. Relative stability of high explosives adducts of chloride, formate, acetate and nitrate using negative ion ESIMS.
Figure 6.4. Characterization of EGDN-formate, m/z 197 and formic acid-formate, m/z 91 adducts at different temperatures (a) and as a function of solution pH (b) using negative ion ESIMS.
E. Method Validation

To validate a new HPLC-ESIMS method, the instrumental set-up using the post column addition of ammonium nitrate and ammonium chloride employed by Zhao and Yinon was used to assess the detection of the high explosives EGDN, NG, PETN, and RDX as chloride and nitrate adducts.\textsuperscript{13} In addition, TNT and HMX were used to demonstrate the simultaneous detection of these explosives. The post column addition of 50\%MeOH/50\% aqueous solution containing 0.5 mM ammonium nitrate and 1 mM ammonium chloride was performed by DLI at 0.015 mL/min. into a tee union after the HPLC column prior to the ESIMS source. This post column arrangement resulted in a solution of 50\% methanol/50\% aqueous 0.05 mM ammonium nitrate and 0.1 mM ammonium chloride at the ESIMS source.

Figure 6.5 shows the extracted ion chromatograms of chloride adducts of EGDN, NG, RDX, and HMX, nitrate adduct of PETN, as well as the deprotonated molecule of TNT. The extracted ion chromatograms for each adduct were used to determine linear calibration parameters using standard mixtures from 1-350\(\mu\text{g/mL}\). Individual limits of detection ($S/N = 3$) for the selected explosives and these two adducts were determined to compare the two instrumental set-ups. The limits of detection determined using HPLC-ESIMS with post column addition are given in Table 6.1. While the post column method demonstrates the detection of high explosive adducts, the addition of ammonium salts of formate and acetate might improve the method selectivity. In addition, the use of these anions in the mobile phase, as opposed to post column addition, would allow for this method to be applied without a complicated instrumental set-up.
Table 6.1. Limits of detection (S/N = 3) using HPLC-ESIMS with post column addition of 50% MeOH/50% aqueous solution containing 0.05 mM ammonium nitrate, and 0.1 mM ammonium chloride.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$m/z$</th>
<th>µg/mL</th>
<th>$m/z$</th>
<th>µg/mL</th>
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<tr>
<td></td>
<td>[M+Cl]$^-$</td>
<td></td>
<td>[M+NO$_3$]$^-$</td>
<td></td>
</tr>
<tr>
<td>EGDN</td>
<td>187</td>
<td>5.5±0.9</td>
<td>214</td>
<td>4.6±0.9</td>
</tr>
<tr>
<td>NG</td>
<td>262</td>
<td>9.2±0.8</td>
<td>289</td>
<td>8.3±0.6</td>
</tr>
<tr>
<td>PETN</td>
<td>351</td>
<td>13.3±0.7</td>
<td>378</td>
<td>13.9±0.2</td>
</tr>
<tr>
<td>RDX</td>
<td>257</td>
<td>7±2</td>
<td>284</td>
<td>8±1</td>
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<tr>
<td>HMX</td>
<td>331</td>
<td>10±1</td>
<td>358</td>
<td>10±2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[M-H]$^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNT</td>
</tr>
</tbody>
</table>
Figure 6.5. Extracted ion chromatograms of standard mixture illustrating the detection of high explosives by negative ion ESIMS. Solutions containing 10\(\mu\)g/mL of all standards and 60\(\mu\)g/mL EGDN was used for HPLC separation using a mobile phase consisting of 50\% MeOH/aqueous 0.05mM ammonium nitrate, and 0.1mM ammonium salts of formate, acetate and chloride.
In the second instrumental procedure, the novel isocratic HPLC method was used for the detection of anionic adducts of EGDN, NG, PETN, RDX, and HMX, as well as the deprotonated molecule of TNT. A mobile phase consisting of 50% MeOH/50% aqueous solution containing 0.05 mM ammonium nitrate, 0.1 mM ammonium chloride, 0.1 mM ammonium formate, and 0.1 mM ammonium acetate was used for the separation of the explosives in standard mixtures. By adding the ammonium salts of nitrate, chloride, formate, and acetate directly to the mobile phase, the analysis of the selected high explosives was accomplished without the need for a separate DLI pump for the anionic additives. Table 6.2 lists the adduct ions of EGDN, NG, PETN, RDX, and HMX as well as the deprotonated molecule of TNT. The calibration data for the high explosives was determined using the extracted ion chromatograms for each adduct. Standard mixtures from 5-350 µg/mL were used to establish the limits of detection (S/N = 3) for the multiple adducts. As given in Table 6.2., the limits of detection using the HPLC-ESIMS method were comparable to those using post column addition. The difference between the limits of detection for all the compounds determined by the two methods was significant as verified by two-way ANOVA (p = 0.0002). The detection of the acetate adducts of NG and PETN was not effective during the HPLC-ESIMS analysis and thus these detection limits were not determined. While the nitrate adduct of NG provided the lowest detection limit, the chloride adduct of PETN gave a significantly better detection limit. For the analysis of EGDN, RDX, and HMX, both chloride and nitrate adducts were more sensitive than formate and acetate adducts. The detection of multiple adducts of the high explosives is shown in Figure 6.6, which illustrates extracted ion chromatograms of
a standard mixture. The extracted ion chromatograms show the sum of intensities for several characteristic explosives adducts as well as the intensity of the deprotonated molecule of TNT. For example, the detection of NG adducts included NG-chloride, \textit{m/z} 262, NG-formate, \textit{m/z} 272, and NG-nitrate, \textit{m/z} 289. These adduct species are shown in the mass spectrum of NG collected during the chromatographic run in Figure 6.7.

Although the retention of TNT and NG were similar, the characteristic peaks identified in Figure 6.7 illustrate that these two compounds can be differentiated.

By directly comparing the two instrumental set-ups, the methods were validated with the same HPLC and ESIMS parameters. These results indicate that the novel method using a mobile phase containing the ammonium salts is similar to the post column addition. The detection of the explosives was accomplished with improved method selectivity. For example, the quantitation of EGDN adducts included EGDN-chloride, \textit{m/z} 187, EGDN-formate, \textit{m/z} 197, EGDN-acetate, \textit{m/z} 211, and EGDN-nitrate, \textit{m/z} 214, which illustrates that a more comprehensive approach for compound identification can be applied to explosives analysis by forensic laboratories.
Table 6.2 Limits of detection using HPLC-ESIMS with a mobile phase of 50% MeOH/50% aqueous solution containing 0.05 mM ammonium nitrate, 0.1 mM ammonium chloride, 0.1 mM ammonium formate, and 0.1 mM ammonium acetate.

<table>
<thead>
<tr>
<th>Compound</th>
<th>m/z</th>
<th>µg/mL</th>
<th>m/z</th>
<th>µg/mL</th>
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<th>µg/mL</th>
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<tbody>
<tr>
<td></td>
<td>[M+Cl]⁻</td>
<td></td>
<td>[M+CHOO]⁻</td>
<td></td>
<td>[M+CH₃COO]⁻</td>
<td></td>
<td>[M+NO₃]⁻</td>
<td></td>
</tr>
<tr>
<td>EGDN</td>
<td>187</td>
<td>3.5 ± 0.2</td>
<td>197</td>
<td>4.0 ± 0.5</td>
<td>211</td>
<td>8.8 ± 0.9</td>
<td>214</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>NG</td>
<td>262</td>
<td>2.4 ± 0.2</td>
<td>272</td>
<td>1.7 ± 0.1</td>
<td>286</td>
<td>n.d.</td>
<td>289</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>PETN</td>
<td>351</td>
<td>0.6 ± 0.1</td>
<td>361</td>
<td>2.2 ± 0.4</td>
<td>375</td>
<td>n.d.</td>
<td>378</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>RDX</td>
<td>257</td>
<td>1.4 ± 0.3</td>
<td>267</td>
<td>2.5 ± 0.4</td>
<td>281</td>
<td>3.3 ± 0.8</td>
<td>284</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>HMX</td>
<td>331</td>
<td>3.4 ± 0.3</td>
<td>341</td>
<td>5 ± 1</td>
<td>355</td>
<td>5.2 ± 0.4</td>
<td>358</td>
<td>3.4 ± 0.2</td>
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<tr>
<td></td>
<td>[M-H]</td>
<td></td>
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<td>TNT</td>
<td>226</td>
<td>3.4 ± 1.0</td>
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</table>
Figure 6.6. Extracted ion chromatograms of standard mixture illustrating the detection of high explosives by negative ion ESIMS. Solutions containing 10µg/mL of all standards and 60µg/mL EGDN was used for HPLC separation using a mobile phase consisting of 50% MeOH/aqueous 0.05mM ammonium nitrate, and 0.1mM ammonium salts of formate, acetate and chloride.
Figure 6.7. Mass spectrum of NG collected during the chromatographic run, which illustrates detection of NG-chloride, \(m/z\) 262, NG-formate, \(m/z\) 272, and NG-nitrate, \(m/z\) 289 adducts. Isocratic HPLC using 50% methanol/50% aqueous solution containing 0.05mM ammonium nitrate, 0.1mM ammonium chloride, 0.1mM ammonium formate, and 0.1mM ammonium acetate.
F. Conclusions

Based on the preferential formation of gas-phase adduct ions, the characterization of common high explosives was accomplished as shown by the detection of chloride, formate, acetate and nitrate adducts. Using different solvent mixtures, the relative stability of the explosive-adduct pairs were determined by evaluating intensity ratios for EGDN, NG, PETN, RDX, and HMX. The intensities of the EGDN-formate and formic acid-formate adduct ions were evaluated at different ESIMS operating temperatures and pH levels. These initial studies suggest that the stability of the EGDN-formate was less than nitrate, chloride, and acetate but was constant up to 150 °C and increased at pH 8.2. The detection of nitrate and chloride adducts of the selected explosives was evaluated using the post column addition of these anionic additives as ammonium salts. Additional specificity and selectivity was illustrated by using the HPLC-ESIMS method for the analysis of explosives with chloride, formate, acetate, and nitrate in the chromatographic mobile phase. The HPLC-ESIMS method developed in this study provides a more comprehensive approach for the analysis and identification of common high explosives.
VII. Chapter 7. Conclusions

While GC-MS and HPLC-MS methods have previously been developed for specific applications, very few can be used for the detection of a wide range of explosives. The development of comprehensive instrumental methods is needed to provide efficient procedures that can be applied to explosives analysis in forensic laboratories. The methods developed in the present studies have been used to quantify and identify several explosives and related compounds. In addition, these investigations deal with the practical aspects of employing the novel methods for the analysis of smokeless powder additives and common high explosives.

A gradient reversed-phase HPLC-ESIMS method was developed to establish a more comprehensive technique to identify and quantify the organic constituents in smokeless powders. Using this method, the separation of several compounds with varying polarity was achieved while the ESIMS was optimized for the detection of protonated molecules in the full scan positive ion mode. The HPLC-ESIMS method was used for the analysis of three classes of powder constituents including isomers of nitroso and nitro derivatives of DPA. Subsequent studies characterized the effects of the solvent properties associated with the gradient elution method. The influence of the ammonium acetate concentration, pH, and percent methanol on the ESIMS responses of selected smokeless powder components was investigated by varying the solution parameters. The effect of the ammonium acetate concentration demonstrates that the electrolyte is required for efficient ionization and the ESIMS intensity was optimal at 2 mM for the selected compounds, except 24DNDPA. The aqueous solution pH, corresponding to the
available protons in solution, did not have a significant effect on the ESIMS intensity of the analytes. The percent methanol was evaluated with both decreasing and constant electrolyte concentrations to demonstrate the affects of droplet stability and ion transfer into the gas phase. While the electrolyte was below the optimal level with decreasing ammonium acetate concentrations, the ESIMS intensity increased with increasing organic content at a total concentration of 2 mM. The influence of changes in the mobile phase composition was also used to relate the response of particular analytes to the mechanisms by which ions are produced during electrospray ionization. The ESIMS responses of the non-polar components were enhanced by maintaining a constant ammonium acetate concentration throughout the linear gradient. Although the solution parameters and analytes were exclusive to this study, the experimental approach can be applied to other methods development investigations. To investigate the variation in organic smokeless powder constituents, the simultaneous determination of the organic additives in unburned powders and pipe bomb samples was accomplished using the revised HPLC-ESIMS method. The HPLC-ESIMS method was used to differentiate the smokeless powders by their additive profile. The detection and identification of the organic constituents allowed for the comparison of several smokeless powders from different manufacturers as well as the same powder from different lots.

The separation and detection of six common high explosives was performed using two GC systems and HPLC-ESIMS operated in the negative ion mode. By using GC with a large bore columns and high flow rates, the degradation of the explosives was minimized. A sensitive screening method using GC/ECD and a selective mass
spectrometry method using GC-MS were developed for the evaluation of explosives levels in public places. The HPLC-ESIMS method developed in this study allowed for some insights to be gained from the preferential formation of gas-phase adduct ions for the detection and identification of common high explosives. The relative stability of the explosive-adduct pairs were determined for chloride, formate, acetate, and nitrate by evaluating intensity ratios of the common high explosives. Using isocratic HPLC-ESIMS, the detection and identification of high explosives was accomplished. The analysis of the high explosives adducts using negative ion ESIMS provides unambiguous structural information for these thermally labile compounds.
VIII. Chapter 8. Discussion and Suggestions for Future Work

In the present studies, GC and HPLC-ESIMS methods were developed to quantify and identify explosives and related compounds to address the drawbacks of previous investigations. These analysis methods provide reliable results for several different compounds. The separation and detection of several smokeless powder additives was accomplished using HPLC-ESIMS. In the course of developing a more comprehensive HPLC-ESIMS method for the analysis of smokeless powders, practical implications regarding the detection technique were addressed by characterizing the solution parameters associated with the gradient elution method. The analysis of high explosives was accomplished by GC/ECD as well as GC-MS and HPLC-ESIMS methods operated in the negative ion mode. The newly developed HPLC-ESIMS method provides the first descriptive study defining adduct stability and multicomponent detection that retains distinctive structural information for explosive identification. While the development of these methods focused on forensic investigations, the research approach could be used for other applications.

The application of the HPLC-ESIMS method in future work would prove useful in the analysis of aged and burned smokeless powders as well as GSR. The quantitation and identification of the organic constituents over time can be used to monitor smokeless powder aging to improve storage conditions. The method could also be used to assess the reactions that occur upon deflagration by analyzing burned smokeless powders. For example, the decomposition of the centralite stabilizers produces nitroaniline based compounds that, unlike the derivatives of DPA, have not been characterized. The
HPLC-ESIMS method would be useful for the detection of these compounds, which would provide additional distinction of different smokeless powder samples. The analysis of GSR using this comprehensive method would allow for the comparison of powder samples from the residue and the weapon. For the HPLC-ESIMS method to be amendable for the analysis of GSR, a new sample preparation technique may be needed. An extraction procedure that provides enhanced preconcentration while mimimizing matrix interferences could improve the method sensitivity. Two sample preparation procedures proposed for the analysis of smokeless powders by HPLC-ESIMS include aqueous extraction followed by non-polar SPE or SPME and aqueous extraction with hollow fiber solvent microextraction as an additional preconcentration step. These schemes would mimimze interferences from non-polar species in the first stage and from polar species in the second stage. Optimization studies for these extraction techniques should include the evaluation of several polar organic solvents for the desorption step using SPE or SPME. In addition, these studies should indicate an appropriate solvent for hollow fiber solvent microextraction that is compatible with HPLC-ESIMS.

Suggested future work on the analysis of high explosives can be arranged in three areas. First, the sample preparation techniques proposed for smokeless powder analysis could be used to improve the sensitivity of the methods for high explosives. Previous studies have applied aqueous extraction followed by SPE and SPME for a limited number of energetic compounds and for specific applications. Other studies illustrated the use of solvent microextraction and hollow fiber solvent microextraction as preconcentration techniques for nitroaromatic explosives and dialkylphthalate acid esters.
from aqueous samples.\textsuperscript{83,84} All the newly instrumental developed methods in this research can be used to evaluate the proposed sample preparation procedures for a wide range of explosives and related compounds in a variety of samples. The other two areas regarding future work are specific to negative ion detection by GC-MS or HPLC-ESIMS.

While the GC-MS method developed for the analysis of explosives in the present work takes advantage of the selectivity afforded by NCI, improvements could be made to provide more sensitive results that are structurally informative. Methods that can be used to enhance sensitivity and selectivity include single ion monitoring and tandem MS detection. These methods make use of the capability of present day MS instruments to isolate specific ions for detection in single ion monitoring or isolation and fragmentation in tandem MS. However, a shortfall of this proposal is that tandem MS requires consistent intensity of high mass ions that can be fragmented.

As discussed previously, certain compounds like nitrate esters, EGDN, NG, and PETN, decompose in the GC injector and ion source, which results in indistinguishable data. This shortcoming would not be a problem for single ion monitoring but optimization of the operating conditions of the tandem MS method could provide reliable results. Using both of these MS modes, the fragmentation of stable explosives such as TNT and RDX could be carried out by tandem MS in the daughter ion mode in addition to the detection of fragmentation products of the nitrate esters by single ion monitoring. Another approach to distinguish the MS and tandem MS data for these thermally labile compounds is to apply multivariate analysis procedures that would resolve minor spectral variations and allow for conclusive explosive identification.\textsuperscript{16}
Future work using negative ion ESIMS for the detection of adducts of explosives and related compounds would be useful to provide fundamental data to improve the understanding of the electrospray process. While the studies in this research were designed to demonstrate the application of negative ion detection by analyzing mixtures of explosives, similar studies using individual explosives and anions could be performed to elucidate specific gas-phase interactions. For example, the gas-phase production of protons from ammonium may be responsible for the neutralization of deprotonated explosives. This hypothesis can be tested by infusing alkylated ammonium salts in the solvent and then adding gas-phase ammonia in the ESIMS source to determine the effect of these reactions.

Additionally, an investigation of the gas-phase thermodynamics involved in the detection of high explosives would be useful to determine the physical properties controlling ionization efficiency. Factors that could be important in the production of explosives adducts include the dipole moments, volatility, and ion mobility of the different substituents present in the electrosprayed solution. The effects of these factors can be evaluated by deriving relationships between the physical properties and the ESIMS intensities of the explosive adducts. In addition, these studies could be supplemented by theoretical investigations using contemporary methods that include solvation parameters. Furthermore, these investigations could also be used for direct investigation of the ion evaporation theory and evaporation partitioning model using ESIMS in the negative ion mode. Because the evaporation partitioning model is constrained by the equilibrium constants of ions between inner and outer regions of
electrosprayed droplets as well as between the droplet surface and the gas-phase, the effects of ionic equilibria and volatility during electrospray ionization are difficult to determine. Negative ion ESIMS studies of explosive-adduct ions could provide some insight to these processes due to the wide range of volatility. An additional possibility for future research to improve explosives analysis is the ESIMS detection of explosives adducts with specific amino acids or oligopeptides found in nitrate reductase or related enzymes.\textsuperscript{85,86} While the selectivity of adduct formation should be suitable for nitrate ester explosives, this detection scheme should also be tested with nitroaromatics and nitramines.

Another approach for future work is to combine the two HPLC-ESIMS methods employed in this research. The development of an all-inclusive procedure could be accomplished with one mobile phase using both negative and positive ionization modes within the same run depending on the retention characteristics desired. Because double based smokeless powders contain NG, the use of both ionization modes in the same chromatographic run would allow for all the major components in these powders to be identified. Because NG is more polar than the phthalates and centralites that were separated by the gradient elution method, the negative ion mode could be used until NG elutes followed by the positive ion mode for the other smokeless powder additives. The effect of the solution parameters associated with the mobile phase should be characterized and optimized to enhance ionization efficiency. The results of the current research could be used as the starting point for these studies. For example, the electrolyte was used to promote ionization in the positive ion mode but several ammonium salts
were used as the source of anions in the negative ion mode. While the ammonium acetate concentration was optimal at 2 mM in positive ion ESIMS detection of smokeless powder additives, the electrolyte level for the negative ion method would need to be optimized between 0.1 to 2 mM. Because the detection of smokeless powder additives in the positive ion mode and the detection of high explosives in the negative ion mode have been demonstrated in the present work, a comprehensive procedure combining these methods would be useful for the identification of explosives samples in forensic laboratories.
References

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Appendix A. Positive Ion ESIMS Mass Spectra of Smokeless Powder Additives

Figure A.1. Positive Ion ESIMS mass spectrum of diphenylamine (DPA, Mr 168).

Figure A.2. Positive Ion ESIMS mass spectrum of dimethyl phthalate (DMP, Mr 195).

Figure A.3. Positive Ion ESIMS mass spectrum of N-nitrosodiphenylamine (NsDPA, Mr 198).

Figure A.4. Positive Ion ESIMS mass spectrum of 4-nitrodiphenylamine (4NDPA, Mr 214).

Figure A.5. Positive Ion ESIMS mass spectrum of diethyl phthalate (DEP, Mr 222).

Figure A.6. Positive Ion ESIMS mass spectrum of methyl centralite (MC, Mr 240).

Figure A.7. Positive Ion ESIMS mass spectrum of ethyl centralite (EC, Mr 268).

Figure A.8. Positive Ion ESIMS mass spectrum of dibutyl phthalate (DBP, Mr 278).
Figure A.1. Positive Ion ESIMS mass spectrum of diphenylamine (DPA, Mr 168).
Figure A.2. Positive Ion ESIMS mass spectrum of dimethyl phthalate (DMP, Mr 195).
Figure A.3. Positive Ion ESIMS mass spectrum of N-nitrosodiphenylamine (NsDPA, Mr 198).
Figure A.4. Positive Ion ESIMS mass spectrum of 4-nitrodiphenylamine (4NDPA, Mr 214).
Figure A.5. Positive Ion ESIMS mass spectrum of diethyl phthalate (DEP, Mr 222).
Figure A.6. Positive Ion ESIMS mass spectrum of methyl centralite (MC, Mr 240).
Figure A.7. Positive Ion ESIMS mass spectrum of ethyl centralite (EC, Mr 268).
Figure A.8. Positive Ion ESIMS mass spectrum of dibutyl phthalate (DBP, Mr 278).
Appendix B. Negative Chemical Ionization Mass Spectra of High Explosives

Figure B.1. Negative Chemical Ionization (NCI) mass spectrum of EGDN, (Mr 152).
Inset, m/z 70-325 enlarged by 50 times.

Figure B.2. Methane NCI mass spectrum of RDX, (Mr 222).

Figure B.3. Methane NCI mass spectrum of NG, (Mr 227). Inset, m/z 70-325 enlarged by 50 times.

Figure B.4. Methane NCI mass spectrum of TNT, (Mr 227).

Figure B.5. Methane NCI mass spectrum of PETN, (Mr 316). Inset, m/z 70-325 enlarged by 50 times.
Figure B.1. Methane NCI mass spectrum of EGDN, (Mr 152). Inset, m/z 70-325 enlarged by 50 times.
Figure B.2. Methane NCI mass spectrum of RDX, (Mr 222).
Figure B.3. Methane NCI mass spectrum of NG, (Mr 227). Inset, m/z 70-325 enlarged by 50 times.
Figure B.4. Methane NCI mass spectrum of TNT, (Mr 227).
Figure B.5. Methane NCI mass spectrum of PETN, (Mr 316). Inset, m/z 70-325 enlarged by 50 times.
Appendix C. Negative Ion ESIMS Mass Spectra of High Explosive Adducts

Figure C.1. Negative ion ESIMS mass spectrum of EGDN-formate adduct, $m/z$ 196.

Figure C.2. Negative ion ESIMS mass spectrum of EGDN-chloride adduct, $m/z$ 186.

Figure C.3. Negative ion ESIMS mass spectrum of NG-chloride adduct, $m/z$ 262 and NG-nitrate adduct, $m/z$ 289.

Figure C.4. Negative ion ESIMS mass spectrum of NG-chloride adduct, $m/z$ 262, NG-formate, $m/z$ 272, and NG-nitrate adduct, $m/z$ 289.
Figure C.1. Negative ion ESIMS mass spectrum of EGDN-formate adduct, m/z 197.
Figure C.2. Negative ion ESIMS mass spectrum of EGDN-chloride adduct, $m/z$ 187.
Figure C.3. Negative ion ESIMS mass spectrum of NG-nitrate adduct, m/z 289
Figure C.3. Negative ion ESIMS mass spectrum of NG-nitrate adduct, $m/z$ 289, and NG-chloride adduct, $m/z$ 262.
Figure C.4. Negative ion ESIMS mass spectrum of NG-chloride adduct, $m/z$ 262, NG-formate, $m/z$ 272, and NG-nitrate adduct, $m/z$ 289.
Appendix D. Supplemental Instrument Instructions and Laboratory Procedures

D.1. Finnigan GCQ GC-MS Tuning Instructions
D.2. Determination of Nitroglycerine in Smokeless Powders
D.3. Analyzing High Explosives Using GC/ECD
D.4. HP GC-MSD Operating and Tuning Instructions
Finnigan GCQ GC-MS Tuning Instructions

Start Tune interface using desktop icon.

Note: Electron Impact positive ion mode (EI+) is the default mode. If instrument or tune software was (re)started, it is already in EI+. If tune is running, set – up EI+ mode in the menu: “Tune” then “Manual”

Tuning Instructions:
Initialize Calibration Gas, wait 15 seconds. Blue icon on tune toolbar or menu item under Instrument Settings
Start Automatic Tune with all boxes checked: “Tune” and “Automatic”
- if any part fails, see manual and take corrective action
- if failure in Resej Ampl calibration occurs, this can be skipped in special situations but only when the resulting tune is good (~1 million Intensity Units)

If EI+ or CI+ will be used in your method; Stop here. Save hard copy of tune report.
Also make electronic copy via “Alt-PrintScreen” as shown below for “EI Source”

Adjust CI gas pressure on the manual adjustment knob on the front of the MS while running the full scan “Experiment” with the calibrant gas on. The recommended pressure on the Ion Gauge is 2.0 to 5.0 x10⁻⁴ Torr.
Tuning Instructions for Negative Chemical Ionization:

Set-up CI negative mode:

Initialize CI Gas, wait 15 seconds

Select Negative Ion Polarity: “Tune” and “Manual”

Start Automatic Tune: “Tune” and “Automatic”

Select Lens tune and Calibration masses (See operators manual, web, or NCItuning.doc for FC-43 negative ion masses) Note: calibration masses depend upon application, for a wide range of m/z’s, i.e.

- If any part fails, see manual and take corrective action
- If Intensity is significantly lower than “best” recent CI- tune report as shown below for “CI Source”, it fails

Save hard copy of tune report. Also make electronic copy, including calibration masses via “Alt-PrintScreen”
Note: if negative CI mode will be used in GC-MS method, leave CI gas and negative ions on when exiting the tune interface. Select negative CI tune and negative ion checkbox in the Instrument Set-up on the MS page.

Figure D.1.1. GCQ EI positive ion mode tune report.
Figure D.1.2. GCQ CI negative ion mode tune report.
Determination of Nitroglycerine in Smokeless Powders

(Version 0)

I. Background.

The analytical techniques for the analysis of explosives are based upon the detection of active ingredient. Smokeless powders are considered low explosives and are used as propellants for ammunition. Prepared mechanically by extrusion and drying, smokeless powders are arranged into classes corresponding to the added energetic material. While single-based powders contain only nitrocellulose; double-based powders consist of both nitrocellulose and nitroglycerine (NG). Smokeless powder analysis is typically performed using organic liquid extraction followed by gas chromatography - mass spectrometry (GC-MS). Mass spectrometry is a detection technique that provides both quantitative and structural information. In this lab, the determination of NG in smokeless powder will be performed using liquid extraction and GC-MS.

![Nitroglycerine](attachment:image.png)
II. Reagents.

Methylene chloride extraction solvent, double-based smokeless powder sample, nitroglycerine standard solution, micropipettes, vials and GC syringe will be provided by the TA.

III. Procedure.

A. Extraction.

Weigh 20mg smokeless powder sample into a clean glass vial containing 1mL methylene chloride, mix by shaking the powder discs to bottom of the vial.

B. NG Standard Solution

Prepare calibration standards of nitroglycerine at 20, 50, and 80 µg/mL (parts per million, ppm) in vials by dilution to a total volume of 100 µL.

C. GCMS

1. Nitroglycerine Standards

a. The TA will set-up the GCMS instrument for chemical ionization in the negative ion mode in the “GCQTune” page - this mode will be shown on the GCQ MS panel by the indicators:

   ION MODE (-) and EI/CI (CI).

b. Start the Xcalibur software.

   Select “Sequence setup”

   Open the “expl_chem487.sld” sequence.
In Line 1 the file name and Inst Method should contain the latest file name and the “0expl Chem487.meth” method.

Change the file name by a Double-click on “File Name” and enter appropriate name with “.RAW” at the end. You may use the same file name for all GCMS measurements-the software will name the first file with the name alone, then subsequent files will be saved with that name and a string of numbers containing the date and time of the run. Add comments as necessary. If needed, double-click on “Inst Method” in Line 1 and select “0expl Chem487.meth”

c. Measure the GCMS response for the NG calibration standards by injecting 1µL with the GC syringe. Record peak areas for all standards. Print a mass spectrum and identify the major peaks.

In the “Sequence setup” choose “Action” and select “Run this Sample” then click “Ok” When the blue “Ready” indicator is shown on the GC panel, press the blue “Start/Stop” button and inject 1µL of the sample with the GC syringe.

In the Xcalibur software, select the “Road Map” view and click on “Qual browser”

Open the data file according to the file name you entered. Select the ‘push pin’ indicator on the top panel to select the chromatogram. Click on “Toggle Peak Detection” on the toolbar. The retention time (RT) and peak area (AA) should now be displayed on the chromatogram (top panel).

Select the ‘push pin’ indicator for the lower panel to select the mass spectrum. Click on the peak to display the MS scan- you may click and drag the width of the peak
to obtain the average mass spectrum. Select print and the “selected cell only” to print the mass spectrum.

2. Smokeless powder sample.
   a. Dilute smokeless powder/methylene chloride extract by adding 10µL extract to 990µL methylene chloride in a clean vial.
   b. Measure GCMS response of the diluted smokeless powder sample using the directions above to determine the peak area and print the mass spectrum.

IV. Calculations

A. Tabulate the peak areas of the nitroglycerine standards. Determine the mean, standard deviation, and 95% confidence intervals for each standard.

B. Plot the calibration curve of peak area vs. concentration. Use least squares procedures to determine the calibration model. Report the values of $R^2$ and standard error estimate for your calibration model.

C. Determine the concentration of nitroglycerine in the smokeless powder sample for each GCMS run. Calculate the mean, standard deviation, and 95% confidence intervals for the three NG concentrations. Using the mean concentration and dilution factors, compute the percent composition in the original weighed sample.

D. Identify the negative ions represented by the major peaks in the mass spectra from your standard and smokeless powder sample.
Analyzing High Explosives Using GC/ECD

A. Explosives Standard Solution

Prepare calibration standards containing ethylene glycol dinitrate (EGDN), nitroglycerine (NG), trinitrotoluene (TNT), pentaerythritol tetranitrate (PETN), cyclotrimethylenetrinitramine (RDX), and cyclotetramethylenetetranitramine (HMX), at 20, 50, and 80 ng/mL (parts per billion, ppb) in vials by dilution with acetone to a total volume of 100 µL.

B. Analysis of Standards

1. Load Chemstation software. (located on Desktop or in Start Menu)
2. Open Method by clicking on “Method”, “Load Method” on the top menu. Choose exp_ecd.m. (Located in c:/hpchem/1/methods)
3. Manually burn out the column and detectors on the GC. Press the oven button on the GC unit, then input 250 on the number pad followed by Enter. The same should be done to the Front Inlet and Back Detector. Allow instrument to heat for at least 10 min.
4. Reload method by following Step 2. When prompted to save changes in method, select “No.”
5. While instrument is cooling, select “Run Control” “Sample Info” from the top menu. On the screen that appears, change Operator Name by entering your initials. Change the subdirectory name if necessary. (This is the folder the data will be stored in.)
*Note that if a new subdirectory is started, the counter must be reset to 0000000. This counter will automatically change for each sequential run. Now enter the sample name in the box marked as such. Use the large box on the bottom to enter any additional comments. Select Ok when complete.

6. When the status bar on the screen has a green “Ready,” press F5. The status bar should then say “Waiting for Injection.”

7. Wash 10uL syringe with acetone a 3-4 times, then move plunger to 1uL mark. Collect 1uL of sample.

8. Press “Start” on GC and immediately inject sample. Be sure to inject sample quickly in one fluid motion. After run is complete, repeat steps 5-8 for remaining samples.

9. Record peak areas for all standards by printing the chromatogram report for each run.

C. Data Analysis

1. Using the reprocessing copy of Chemstation, which is found under the “View” menu, load each chromatogram in “data analysis.” Ensure all peaks of interest are integrated. Under the “File” menu choose “Export” as csv. On the menu that appears, choose the option to ‘copy to the clipboard.” Click Ok on the next two menus that appear. Paste the data into Microsoft Excel and label each column manually. (time, area, width, height)
2. Calculate the retention factor for each peak, $k' = (t_r - t_0)/t_0$, where; $t_0$ is the retention time of the unretained peak and $t_r$ is the retention time of the peak of interest.

3. Plot the calibration curve of peak area vs. concentration. Use least squares procedures to determine the calibration model. Report the values of $R^2$ and standard error estimate for your calibration model.

D. Additional Data Analysis

Export the chromatogram picture on the screen in “data analysis” by choosing “Graphics” on the Chemstation menu and copy to the clipboard. Paste in Microsoft Word or Excel for reporting.
HP GC-MSD Operating and Tuning Instructions

Start-up

Ensure rough vacuum pump oil is filled to appropriate level and hosing is plumbed correctly.

Check column connections inside GC oven, check seal on transfer line to MS.

Switch Power “on” at rear of MS. Allow diffusion pump to reach operating temperature, ca. 20 min.

Start computer and Chemstation Software; GC_MS Instrument #1 Icon on Desktop. GC instrument will load the current method, i.e. injector, oven, detector temperatures. Check DET B Temp on GC control panel, set to 280 °C.

On window “File” menu, select “View” and “Diagnostics/Vacuum Control” Select “Vacuum” and “Vacuum Status” If not ready, pop-up warnings will notify user that ~ 4 hours are needed to pump down instrument. When “Ready to Run” Readings should be: Diffusion pump, Heater on, Hot MS Source: > 100 degC Foreline Pressure: 20-40 mTorr, Pressure OK

Select OK to exit.
Tuning Instructions

Select “View” and “Manual Tune”

Select “File” and “Load Tune Values” and select latest known “good” tune. If unknown, using Window Explorer, view hard drive contents of tune values at C:\HPCHEM\5971 and select last saved tune.

Select “Tune” and “User Tune”

If tune fails, see Help and web references. Reload saved tune values. Check masses, mass range, and other Acquisition parameters under “AdjParam” and “EditAcq Params”

If changes are necessary, select “AdjParam” and “EditMS Params” and click “Prof” (profile scan) and check for mass accuracy and intensity Ab (absolute intensity, first peak >100,000 to 1 million, and last peak >1000), “Stop” profile scan. Adjust further using “MoreParams” and “AcqParams”. Click “Ok”

Repeat User Tune until satisfactory. Hardcopy of the Tune values will be printed.

Select “View” and “Instrument Control to return to Operating page”
Operating Instructions

Select “Method” and “Load” to use saved method file. Ensure current tune file is being used by clicking on “MS” icon, “Select MS Tune File”

To set-up new method, adjust GC and MS settings.

Click on “Oven” and select “Edit GC Temperatures”

Choose Injector, Detector ‘Zone Temperatures’

Build ‘Oven Program’ by selecting Initial temperature, init. time, and ‘levels’ of temperature program, Rate (range: 1-40 °C/min.), choose final temp. and final time. If level is not needed input 0 rate. Total run time will be calculated.

Click on “MS” and Edit MS SIM/Scan” to adjust parameters.

Choose Solvent delay, >0.5min. to allow solvent peak to pass MS prior to collecting data; this will prolong the lifetime of instrument.

Select Acq. (Acquisition) Mode and MS parameters using desired Scan range or SIM (single ion monitoring) settings. Note: Scan range must be included in Tune file values.

Save Method by Selecting “Method” and “Save”

Click on “Sample Name:”

Select ‘Operator Name, Data File Name:, Sample Name:, and Misc. Info’
Vial Number: 1 is default setting for manual injection.

Data File Name must be changed for each sample’s data to be stored.

Select “Start Run”

Dialog will appear after method parameters are loaded: ‘Acquisition- Prepare to Inject’

Follow instructions.

Note: DO NOT PUT WATER IN GC INSTRUMENTS.

Injection volume should be between 0 to 5microliter, (typically 1? L, for 50-100microgram/milliliter concentration). Adjust Injection volume according to concentration and dilute samples in organic solvent as necessary.

Inject sample and click “Start”

Note: Ways to start run: Click Start with mouse; Hit enter on Keyboard; or Press Start on GC Instrument Panel.

View Progress of Run in ‘Total Ion’ window, adjust view with up/down arrows.

Select “View” and DataAnalysis (offline)”

Click “File” and “Load Data File” Choose ‘Data File Name’ for selected data file.

View spectrum by using right double click on chromatogram.

User may zoom using left mouse button to select region of chromatogram or spectrum.

To return to full view, use left double click.
Select paper orientation in “File” and “Printer Setup”

Select “File” and “Print”; choose window or TIC, Signals, and Spectrum.

References:

http://www.jeol.com/ms/docs/pftba.html

http://www.chem.cornell.edu/aba1/GCms2.html


http://depts.washington.edu/spectral/pages/documentation/ms_msd2.html
Appendix E. Analysis of Variance Tables

Analysis of Variance (ANOVA) Introduction

Table E.1. Format of tabulated ANOVA output.

Table E.2. Comparison of HPLC-ESIMS method sensitivities (Counts/mL/µg) for the analysis of smokeless powder additives using the positive ion mode. See pp. 95 and Figure 4.10.

Table E.3. Evaluation of limits of detection (µg/mL) two GC-MS columns for high explosives. See pp. 115 and Table 5.2.

Table E.4. Effect of the solution pH on the intensities of the EGDN-formate and formic acid-formate adducts. See pp. 126 and Figure 6.4b.

Table E.5. Comparison of HPLC-ESIMS method limits of detection (µg/mL) for the analysis of high explosive adducts using the negative ion mode with two instrumental set-ups. See pp. 126, pp. 130, pp. 134, and Table 6.1 and Table 6.2.
Analysis of Variance (ANOVA) Introduction

Analysis of variance (ANOVA) is used to investigate the effect of various factors on a particular response. ANOVA determines the relationship between the factors (also known as treatments) and the response by defining the different sources of variation. The variations derived from ANOVA are compared to that which is expected by random error. ANOVA allows for the assessment of different types of hypothesis testing such as those used in experimental design and regression. For instance, ANOVA expands the two-sample *t-test* for evaluating the equality of two means to a more general null hypothesis of comparing the equality of more than two means as opposed to the alternate hypothesis of the means not all being equal.

Different ANOVA applications can be used in a variety of situations. Two-way, also called two-factor ANOVA is used when evaluating the effects of two factors. Two-way ANOVA was used in the present research. The two factors were the different compounds and the different solution or instrumental parameters. The responses listed with each parameter: comparison of method sensitivities with different electrolyte levels in the mobile phase, ESIMS adduct intensities at different pH levels, and limits of detection using two instrumental systems, i.e. two different columns in GC-MS and two different mobile phase introduction arrangements using negative ion ESIMS. Microsoft Excel (Ver. 10, 2002), Minitab (Ver. 13.31), and Matlab (Student Ver., PLS Toolbox) software packages were used to tabulate and compute the following ANOVA tables. In each evaluation, the null hypothesis was specified as all responses are equal and tested at the 95% confidence level.
Table E.1. Format of tabulated ANOVA output.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>F calculated</th>
<th>Probability of Equality</th>
<th>F critical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor 1</td>
<td>SS₁</td>
<td>df₁</td>
<td>SS₁/df₁</td>
<td>MS₁/MSₑ</td>
<td>a.</td>
<td>b.</td>
</tr>
<tr>
<td>Factor 2</td>
<td>SS₂</td>
<td>df₂</td>
<td>SS₂/df₂</td>
<td>MS₂/MSₑ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>SSₑ</td>
<td>dfₑ</td>
<td>SSₑ/dfₑ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>SSₖ</td>
<td>dfₖ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Computed from F distribution using confidence level and degrees of freedom. Probability of a type I error (rejecting a true hypothesis).
b. Tabulated value at particular confidence level and degrees of freedom. If $F_{calc} > F_{crit}$, reject null hypothesis; probability < alpha level (alpha level is 100 minus confidence level)

Table E.2. Comparison of HPLC-ESIMS method sensitivities (Counts/mL/µg) for the analysis of smokeless powder additives using the positive ion mode. See pp. 95 and Figure 4.10.

Two-way ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>5.08x10¹⁴</td>
<td>7</td>
<td>7.25x10¹³</td>
<td>6.78</td>
<td>0.01</td>
<td>3.78</td>
</tr>
<tr>
<td>Electrolyte Concentration Error</td>
<td>1.03x10¹⁴</td>
<td>1</td>
<td>1.03x10¹⁴</td>
<td>9.61</td>
<td>0.02</td>
<td>5.59</td>
</tr>
<tr>
<td>Error</td>
<td>7.49x10¹³</td>
<td>7</td>
<td>1.07x10¹³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.85x10¹⁴</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table E.3. Evaluation of limits of detection (μg/mL) two GC-MS columns for high explosives. See pp. 115 and Table 5.2.

Two-way ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>14.4</td>
<td>4</td>
<td>3.6</td>
<td>0.1</td>
<td>0.94</td>
<td>6.4</td>
</tr>
<tr>
<td>GC Column</td>
<td>384.4</td>
<td>1</td>
<td>384.4</td>
<td>17.9</td>
<td>0.01</td>
<td>7.7</td>
</tr>
<tr>
<td>Error</td>
<td>85.6</td>
<td>4</td>
<td>21.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>484.4</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table E.4. Effect of the solution pH on the intensities of the EGDN-formate and formic acid-formate adducts. See pp. 126 and Figure 6.4b.

Two-way ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adducts</td>
<td>4.65x10^5</td>
<td>4</td>
<td>1.16x10^5</td>
<td>0.23</td>
<td>0.91</td>
<td>6.39</td>
</tr>
<tr>
<td>pH</td>
<td>1.06x10^7</td>
<td>1</td>
<td>1.06x10^7</td>
<td>20.88</td>
<td>0.01</td>
<td>7.71</td>
</tr>
<tr>
<td>Error</td>
<td>2.03x10^6</td>
<td>4</td>
<td>5.07x10^5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.31x10^7</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table E.5. Comparison of HPLC-ESIMS method limits of detection (µg/mL) for the analysis of high explosive adducts using the negative ion mode with two instrumental set-ups. See pp. 126, pp. 130, pp.134, and Table 6.1 and Table 6.2.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compounds</td>
<td>77.77</td>
<td>5</td>
<td>15.55</td>
<td>2.47</td>
<td>0.06</td>
<td>2.603</td>
</tr>
<tr>
<td>Method</td>
<td>243.75</td>
<td>5</td>
<td>48.75</td>
<td>7.74</td>
<td>0.0002</td>
<td>2.603</td>
</tr>
<tr>
<td>Error</td>
<td>157.50</td>
<td>25</td>
<td>6.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>479.02</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Vita

John A. Mathis grew up in Gadsden, Alabama. He attended Gadsden State Community College, where he was the President of the Science, Math, and Engineering Club. John spent four years active duty with the United States Marine Corps. Before his honorable discharge in 1997, he was stationed in Cherry Point, North Carolina and Okinawa, Japan and received the Navy Achievement and Good Conduct medals. John received B.S. Biochemistry and M.S. degrees from East Carolina University, Greenville, North Carolina. While working under the direction of Yu Yang, he performed analytical chemistry research on subcritical water in sample preparation and separation techniques. John completed a Ph.D. in analytical chemistry with Bruce McCord at Ohio University. His research made use of liquid chromatography-electrospray ionization mass spectrometry and focused on the development of more comprehensive methods for the analysis of explosives and related compounds. John accepted position as a post doctoral analytical chemist at Noramco Inc., a Johnson & Johnson Company in Athens, GA.