SOLVING PROBLEMS IN ION MOBILITY MEASUREMENTS OF FORENSIC
SAMPLES WITH THERMAL DESORPTION AND DYNAMIC MODELING

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Fast screening procedures are necessary when forensic samples are being investigated, which is apparent when screening for explosives, narcotics, and bacteria. Many forensic problems can be solved by traditional analytical methods such as gas chromatography (GC) and liquid chromatography (LC). However, ion mobility spectrometry (IMS) offers several advantages that enhance its applicability as a screening procedure compared to traditional methods. Some of these advantages include its high sensitivity, low detection limits, rapid response, portability, and real-time monitoring capabilities. SIMPLE-to-use Interactive Self-modeling Mixture Analysis (SIMPLISMA) allows temporal information in the data to be exploited so that information is not lost by averaging.

Several methods were developed to produce systems that could solve forensic problems rapidly. First, by utilizing a temperature ramped thermal desorption IMS system with SIMPLISMA, interfering compounds were separated from explosives. Without the ramped thermal desorption, separation of the different compounds was not possible. Also, coupling solid phase extraction (SPE) with IMS allows for the detection of trace levels of analytes in liquid matrices. The detection of explosives in water is very important because 2,4,6-trinitrotoluene (TNT), cyclotrimethylene trinitramine (RDX),
and cyclotetramethylene tetranitramine (HMX) are toxic to humans. By using SPE-IMS, detection and identification of the explosives in water was achieved, possible interferences were removed, and very low detection limits were obtained. Also, the detection of illegal narcotics and their metabolites in urine is important for workplace drug screening. The advantages of utilizing SPE-IMS for these samples include simultaneous detection of several possible compounds and removal of adulterants that might interfere with detection of the narcotics. Finally, adding a thermal hydrolysis/methylation (THM) step to IMS provides a method to detect and identify bacteria rapidly by their fatty acid methyl ester profiles. By utilizing THM-IMS, *Bacillus cereus* and three different Listeria bacteria could be detected and identified rapidly. SIMPLISMA and principal component analysis (PCA) were utilized to classify the data.

Solving problems in ion mobility measurements of forensic samples is possible by IMS without utilizing more complex systems such as GC-IMS or LC-IMS which reduce its applicability as a rapid screening technique.
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This analysis was conducted isothermally at 300 °C. Components are ordered by SIMPLISMA purity value.

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This analysis was conducted isothermally at 300 °C. Components are ordered by SIMPLISMA purity value.

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**Figure 4.13** SIMPLISLMA extracted spectra of THM *Listeria monocytogenes* whole cells. This analysis was conducted isothermally at 300 °C. Components are ordered by SIMPLISMA purity value.

**Figure 4.14** Ion mobility spectral scores showing 60.2% of the cumulative variance between the first two principal components of THM bacteria SIMPLISMA results.

(B) *Bacillus cereus*, 5 experiments; (I) *Listeria innocua*, 5 experiments; (S) *Listeria seeligeri*, 5 experiments; (M) *Listeria monocytogenes*, 5 experiments. Each experiment consisted of placing bacterial whole cells on an IMS filter disk, adding 1 µL of TMAH, and analyzing by thermal desorption IMS. Each sample of bacteria was also taken from a different plate to ensure reproducible results. From this figure, the bacteria are clustered.
List of Abbreviations

IMS .............................................................. ion mobility spectrometry
CAM ............................................................... chemical agent monitor
SIMPLISMA ....................................................... SIMPLE-to-Use Interactive Self-
modeling Mixture Analysis
SPE .................................................................. solid phase extraction
THM ................................................................. thermal hydrolysis/methylation
DM ................................................................. detector module
PPM ................................................................ power/pump module
DCPM ............................................................... DC power module
TOF ................................................................. time-of-flight
APCI ................................................................ atmospheric-pressure chemical
ionization
$K_0$ ................................................................. reduced mobility
TNT ................................................................. 2,4,6-trinitrotoluene
PV ................................................................. pure variable
2D ................................................................. two-dimensional
FAA ............................................................... Federal Aviation Administration
RDX ............................................................... cyclotrimethylene trinitramine
PETN .............................................................. pentaerythritol tetranitrate
HMX ............................................................... cyclotetramethylene tetranitramine
PETN-H .................................................................proton abstraction of PETN
PETN-Cl .................................................................chloride adduct of PETN
PETN-NO₃ ...............................................................nitrate adduct of PETN
PETN-F .................................................................fingerprint oil adduct of PETN
HMX-H .................................................................proton abstraction of HMX
HMX-Cl .................................................................chloride adduct of HMX
HMX-NO₃ ...............................................................nitrate adduct of HMX
2HMX-Cl ...............................................................HMX dimer-chloride adduct
LabVIEW ............................................................Laboratory Virtual Instrument Engineering Workbench
FBI .................................................................Federal Bureau of Investigation
3D .................................................................three-dimensional
RIP .................................................................reactant ion peak
CERCLA ...............................................................Comprehensive Environmental Response, Compensation, and Liability Act
SDB-RPS ..............................................................poly(styrenedivinylbenezene)-reverse-phase sulfonated
C-8 .................................................................octyl
C-18 .................................................................octadecyl
RDX-Cl ...............................................................chloride adduct of RDX
RDX-NO₃ ..............................................................nitrate adduct of RDX
RDX-F.................................................................fingerprint oil adduct of RDX
2RDX-Cl.............................................................RDX dimer-chloride adduct
(-)-Δ⁹-THC.........................................................(-)-Δ⁹-tetrahydrocannabinol
NHSDA..............................................................National Household Survey on Drug Abuse
SAMHSA............................................................Substance Abuse and Mental Health Services Administration
DHHS...............................................................Department of Health and Human Services
TMAH...............................................................tetramethylammonium hydroxide
FAMEs..............................................................fatty acid methyl esters
PCA.................................................................principal component analysis
FSIS...............................................................Food Safety and Inspection Service
PFGE...............................................................pulsed-field gel electrophoresis
Chapter 1. Introduction

1.1. General Statement

Ion mobility spectrometry (IMS) was first introduced in the late 1960s as plasma chromatography, an instrumental technique for detecting organic compounds at trace concentrations in air.\(^1\) Interest in this technique was aroused by its low detection limits, the speed of its response, and its applicability to numerous organic functionalities. F.W. Karasek discussed IMS in a 1974 report reviewing the development of mobility theory and the emerging analytical applications of IMS. At the time, approximately thirty publications were in print reporting exploratory studies in the field of IMS.\(^2\) Twenty-five of these publications were from Karasek and his colleagues describing the applicability of IMS for the detection of trace quantities of gaseous organic compounds at atmospheric pressure. Some of these articles included the detection and identification of alcohols\(^3\), alkyl halides\(^5\), halogenated aromatics\(^6,7\), alkanes\(^8\), nitrosamines\(^9\), alkyl acetates\(^10\), and alkyl amines\(^11\) by IMS. In the mid-1970s, Karasek and his colleagues also investigated the possibility of utilizing IMS for the detection of explosives\(^12\) and narcotics\(^13,14\) vapors, almost twenty years before IMS would become a routine analytical instrument for screening of similar samples. The advantages of IMS are the same today as they were in the 1970s; ion mobility spectrometry offers high sensitivity, instrumental simplicity, low cost, analytical flexibility, and real-time monitoring capabilities.\(^15\)

After the initial surge of work conducted utilizing IMS, it suffered a decline of interest in the late 1970s and early 1980s. This decline was attributed to a broad
disenchantment from unmet expectations and misunderstanding of response characteristics. Early laboratory studies mostly involved single component chemical systems. When multi-component chemical systems were investigated, compounds often interfered with the ionization processes of the target compound and frequently masked its presence completely. Another serious problem associated with IMS is the reliability of quantification; competitive ionization and saturation processes distort the quantitative determination of the target compound. Therefore, ion mobility spectrometry is strictly limited to the introduction of trace quantities of a compound. Large sample sizes tend to saturate the instrument completely depleting the reactant ions. Ion mobility spectrometer detectors also have limited linear ranges as a consequence of the charge transfer equilibria. Also, another disadvantage of IMS is its low resolution. Thus, the potential of IMS as a separation device was deficient compared to other high resolution separation methods, such as gas chromatography (GC) and liquid chromatography (LC). Although its sensitivity was superior to most other analytical methods, the limited peak capacity led many researchers to return to traditional analytical methods.

A new cycle of interest began in the mid-1980s which has resulted in advances in all aspects of IMS including improvements in ionization methods, drift tube technologies, and the usage of reactant ions to increase specificity. Once it was realized that IMS could not resolve all analytical problems, development of single purpose instruments began. This interest primarily occurred through developmental programs within military establishments of the United States of America and the United Kingdom. The three main areas of interest were the detection of chemical warfare agents, explosives, and
proscribed narcotics. The U.S. utilized a “chemical agent monitor” (CAM) manufactured by Graseby Dynamics, Ltd. (Watford, U.K.) extensively during the Gulf War. The CAM is based on IMS technology for the detection of nerve and blister agents.\textsuperscript{19} Ion mobility spectrometers are used in airports throughout the U.S. for both the detection of explosives and narcotics.\textsuperscript{20}

Unfortunately, contemporary IMS analyzers are still insufficient at ionizing and separating all ions in mixtures.\textsuperscript{20} Therefore, separating a sample into individual constituents and introducing them singly into the IMS inlet is desirable. This has resulted in IMS being coupled as a detector to several other analytical techniques such as gas chromatography (GC)\textsuperscript{21-28}, liquid chromatography (LC)\textsuperscript{29-30}, supercritical fluid chromatography (SFC)\textsuperscript{31}, and capillary zone electrophoresis (CZE)\textsuperscript{18}. Ion mobility spectrometry has also been coupled, as the separation device, to mass spectrometers (MS).\textsuperscript{32-36} Hyphenated methods have improved the ionization and separation of components in a mixture, increasing the overall selectivity of the method. However, these methods also increase both the duration of the measurement and the cost of the instrumentation. The ability to transport GC-IMS or IMS-MS systems for on-site detection is also limited. Therefore, solving forensic problems where mixtures could be present without utilizing complex instrumentation which would increase the duration and cost of the experiment would be beneficial.

The basis of this dissertation involves research conducted that solved forensic problems by utilizing IMS with thermal desorption and SIMPLe-to-use Interactive Self-modeling Mixture Analysis (SIMPLISMA). Methods were developed that improved
both the selectivity of measurements and detection limits for the use of IMS as a screening procedure. Samples investigated were in both solid and liquid forms. The methods developed allowed these problems to be solved without sacrificing large amounts of time or the portability of the instrument.

This dissertation presents research conducted on both analytical instrumentation and method development for solving forensic problems by ion mobility spectrometry. The general introduction to IMS and SIMPLISMA are given in this chapter. The development of a temperature ramped thermal desorption ion mobility spectrometer will be presented in Chapter 2. Detection of trace levels of explosives in aqueous solutions and illegal narcotics and their metabolites in urine by solid phase extraction ion mobility spectrometry (SPE-IMS) will be given in Chapter 3. A new technique developed to detect, identify, and classify bacteria by thermal hydrolysis/methylation ion mobility spectrometry (THM-IMS) is presented in Chapter 4. Finally, the overall conclusions and future work will be given in the last chapter. The resulting papers and presentations will be shown in Appendix A and B, respectively.

1.2. Ion Mobility Spectrometry

Ion mobility spectrometry refers to the detection and characterization of chemical substances from their gas-phase ion mobilities. Ion mobilities are determined from ion velocities that are measured in a drift tube. A picture and schematic of the Barringer Ionscan® Model 350 ion mobility spectrometer are given in Figure 1.1 and Figure 1.2, respectively. In Figure 1.1, the detector module (DM) contains the ion mobility
Figure 1.1 Barringer Ionscan® Model 350 with (A) the detector module (DM), (B) the AC power supply and air purifier (PPM), and (C) the standalone DC power supply (DCPM). The Barringer Ionscan® Model 350 was the ion mobility spectrometer used in this research.
Figure 1.2  Schematic of Barringer Ionscan® Model 350 ion mobility spectrometer adapted from reference 16. The arrow indicates the direction of the sample carrier flow gas, and the circles indicate the formation of different ions that are separated based on size and charge in the drift tube.
spectrometer and the user control panel. The AC power supply and air purification system (PPM) are housed in the middle module. Air is purified and dried by activated charcoal and drierite in the air purification system. The Ionscan® Model 350 also has a standalone DC power supply (DCPM) for on-site detection when portability is necessary.

Figure 1.2 is a schematic of the ion mobility spectrometer. The sample under investigation is placed on a sample filter and inserted into a sample cartridge. Figure 1.3 gives a picture of the spectrometer detector inlet system. The sample cartridge is placed in the sample cartridge slide assembly. The assembly is then slid to the left. The desorber anvil rises and forms a closed system with the desorber heater, the sample, and the spectrometer detector inlet. This prevents contamination from room air during an analysis. Referring back to Figure 1.2, the sample is then heated by the desorber heater and vaporized. The vaporized sample enters the reaction region of the spectrometer on a stream of sample carrier gas. A charge transfer cascade produces reactant ions that transfers charge to the analytes. A $^{63}$Ni beta emitter initiates the cascade, and both positive and negative ions are formed. The radioactive source is referred to as the ionizing source in Figure 1.2. When the detection of positive ions is desired, the ion shutter grid and counter-current drift gas inhibit negatively charged ions and neutral molecules from entering the drift region of the spectrometer. The ion shutter grid also prevents positively charged ions from entering the drift tube until it is opened. The shutter has a positive potential when closed and a ground potential when it is open. The repelling rings direct the positive ions through the shutter into the drift tube where the mobility is measured. When the detection of negative ions is desired, the ion shutter grid
Figure 1.3 Barringer Ionscan® Model 350 ion mobility spectrometer detector inlet system. The sample to be analyzed is placed on the (A) sample cartridge slide assembly. The assembly is slid to the left, and the (B) desorber anvil rises to form a closed system with the (C) desorber heater, the sample, and the (D) IMS detector inlet. This prevents contamination in the spectrometer from room air during an analysis.
and counter-current drift gas inhibit positively charged ions and neutral molecules from entering the drift region of the spectrometer. The ion shutter grid also prevents negatively charged ions from entering the drift tube until it is opened. The shutter has a negative potential when closed and a ground potential when it is open. The repelling rings direct the negative ions through the gate and into the drift tube where the mobility is measured. When the ions travel through the drift tube, they will separate based on their different mobilities. The ion size and charge are the primary factors that influence the separation. As the ions are collected at the detector plate, the resultant current is measured. The current as a function of the drift time is the ion mobility spectrum. Shape, polarizability, and charge, as well as the formation of cluster and metastable ions affect the velocity of the ions as they traverse the drift region. The time that each ion exits the drift tube, or the transit time of the ions, is termed the drift time.

Ion mobility spectrometry is similar to time-of-flight (TOF) mass spectrometry in principle; however, there is a major difference in that all IMS processes occur at greater pressures. This forms the basis for the use of IMS for chemical analyses. These advantages include simple and reliable instrumentation, relatively low cost, high sensitivity, real-time monitoring capabilities, short analysis times, and detection at parts per billion concentrations. Charge transfer occurs through atmospheric-pressure chemical ionization (APCI) processes, and the ions that are produced are generally robust, long-lived, and low energy species. For the production of negative ions, three possible reactions may occur. These include an ion transfer reaction (1.1), a charge transfer reaction (1.2), or a dissociative charge transfer reaction (1.3).
A + (HOC₆H₄COOCH₃)ₙ⋅O₂ ⇌ A⋅O₂ + nHOC₆H₄COOCH₃; n=1-3 \hspace{1cm} (1.1)

A + (HOC₆H₄COOCH₃)ₙ⋅O₂ ⇌ A⁻ + O₂ + nHOC₆H₄COOCH₃; n=1-3 \hspace{1cm} (1.2)

A-X+(HOC₆H₄COOCH₃)ₙ⋅O₂ ⇌ X⁻ + A + O₂ + nHOC₆H₄COOCH₃; n=1-3 \hspace{1cm} (1.3)

In the above reactions, methyl salicylate, HOC₆H₄COOCH₃, is the internal calibration gas used when using the Ionscan® Model 350 in negative ion, or explosives, mode. The A is the sample or the analytes under investigation, X is an electronegative moiety of the molecule, and O₂⁻ is superoxide. In addition to the use of a calibration gas in explosives mode, a reactant substance, hexachloroethane, is also used to completely suppress signals from materials that have a weaker electron affinity (i.e. interferents) than chloride. In explosives mode, the sample carrier gas is doped with the hexachloroethane before it is passed above the desorber. The desorbed explosive molecules that contain nitro groups selectively form adduct ions with the chloride ions. This reaction increases the probability of accurate identification of the explosives and lowers detection limits.³⁷

For the production of positive ions, proton transfer is one charge transfer mechanism (1.4).¹⁶

A + H⁺(C₅H₄NCONH₂) ⇌ AH⁺ + C₅H₄NCONH₂ \hspace{1cm} (1.4)

In the above reaction, nicotinamide, C₅H₄NCONH₂, is the internal calibration gas employed when using the Ionscan® Model 350 in positive ion, or narcotics, mode, and once again, the A is the sample or analytes under investigation.³⁷
A consequence of atmospheric pressure chemical ionization is that molecules form ions that retain the conformation and structure of the neutral molecule. At ambient temperatures, product ions seldom dissociate or fragment in the reaction region of the spectrometer because the energetics of APCI processes are weak. When multiple peaks occur for one compound, there are several possible explanations for these instances. First, there is always the possibility of contamination from outside sources. Thermal decomposition of the analyte under investigation is also possible. The decomposition usually occurs prior to the introduction of the sample into the reaction region. For example, decomposition of compounds can occur during the initial desorption of the sample from the filter because high temperatures are employed. Finally, cluster ion formation is possible in which several molecules form intermolecular clusters that captures charge. For example, dimer ions form at higher concentrations. A dimer ion is an intermolecular cluster of two analytes that share a charge. Reaction (1.5), below, describes a dimerization of the analyte molecule.\(^\text{16}\) The formation of dimers has proven to be important for the detection of analytes of interest especially when interferents affect the detection of the monomer. One example is the detection of methamphetamine in the presence of nicotine.\(^\text{38}\)

\[
AH^+ + A \rightleftharpoons A_2H^+ 
\]  

(1.5)

Reduced mobilities \((K_0)\) are often used instead of drift time for identification purposes to correct for instrumental variations. These could include drift potential and drift tube length as well as pressure and temperature of the drift region. Reduced
mobilities provide qualitative results that may be used for identification and help to compare results between instruments, laboratories, and different days.

The reduced mobility \((K_0)\) is expressed by equation (1.6):

\[
K_0 = \left(\frac{v_d}{E}\right) \left(\frac{p_{\text{std}}}{p}\right) \left(\frac{t}{t_{\text{std}}}\right)
\]

(1.6)

for which \(v_d\) is the ion velocity obtained by dividing the drift length by the ion’s transit time, \(E\) is the potential drop (V/cm), and the other two factors correct the drift gas density to STP conditions.\(^1\)

Reduced mobilities of unknown peaks can then be calculated by using the internal calibrants and equation (1.7).

\[
K_0^{\text{unknown}} = \frac{K_0^{\text{calibrant}}}{t^{\text{unknown}}} \cdot t^{\text{calibrant}}
\]

(1.7)

In equation (1.7), \(K_0\) is the reduced mobility in units of \((\text{cm}^2\text{V}^{-1}\text{s}^{-1})\), and \(t\) is the drift time of the calibrant and the unknown.\(^{16}\)

Reduced mobilities were calculated for negative ion mode using methyl salicylate as the calibrant ion. The \(K_0\) of the calibrant ion, which corresponds to the methyl salicylate peak, is calculated by running a sample 1 \(\mu\)L of 2,4,6-trinitrotoluene (TNT) at a concentration of 6.0 mg/L in deionized water, which has an experimental \(K_0\) of 1.45 cm\(^2\)V\(^{-1}\)s\(^{-1}\). The \(K_0\) for TNT according to the literature is 1.54 cm\(^2\)V\(^{-1}\)s\(^{-1}\). To convert from the experimental \(K_0\) used in this work to the literature \(K_0\) values, the ratio of the literature \(K_0\) for TNT to the experimental \(K_0\) for TNT is multiplied by the experimental \(K_0\) for the
substance being studied. Figure 1.4 gives a negative ion average spectrum of the methyl salicylate and superoxide at drift times of 13.2 and 10.3 ms, respectively. This figure corresponds to a blank experiment because no sample was analyzed. The ordinate corresponds to the intensity of the spectrum in volts, while the abscissa corresponds to the drift time measurement in milliseconds. The upper abscissa corresponds to the $K_0$ measurements. In this figure, the $K_0$ of the methyl salicylate is $1.54 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$. This value may differ slightly throughout this dissertation due to slight changes in temperature, humidity, or pressure. The instrument was calibrated with TNT on a daily basis when negative ion mode experiments were conducted.

Reduced mobilities were calculated for positive ion mode using nicotinamide as the calibrant ion. The $K_0$ of the reactant ion, which corresponds to the nicotinamide peak, is calculated by running a sample of 1µL of cocaine at a concentration of 1.0 mg/mL in methanol, which has an experimental $K_0$ of $1.16 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$. Figure 1.5 gives a positive ion average spectrum of the nicotinamide at a drift time of 9.50 ms. This figure corresponds to a blank experiment because no sample was analyzed. In this figure, the $K_0$ of the nicotinamide is $1.86 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$. Once again, this value may differ throughout this dissertation due to changes in temperature, humidity, or pressure. The instrument was calibrated daily with cocaine when positive ion mode experiments were conducted.

1.3. SIMPLE-to-use Interactive Self-modeling Mixture Analysis

In the conventional IMS measurement, the spectra are collected and averaged to yield a single spectrum for each sample. The instrument uses this average spectrum to
Figure 1.4 Average negative ion spectrum of methyl salicylate and superoxide collected on the Barringer Ionscan® 350. The methyl salicylate and superoxide are present whenever a sample is not being analyzed, and the methyl salicylate is the internal calibrant for negative ion mode.
Figure 1.5 Average positive ion mode spectrum of nicotinamide collected on the Barringer Ionscan® 350. The nicotinamide is the internal calibrant for positive ion mode, and it is present whenever a sample is not being analyzed.
identify substances present in the sample. This approach loses important temporal information that occurs during the measurement period. Using chemometric methods, the changes in the spectrometer response can be exploited over time to increase both the sensitivity and selectivity of the measurement. If the sample is altered during the course of the measurement, chemometric methods such as multivariate curve resolution may be used to process the data. Ion mobility spectrometry spectral data often represent mixtures of several components that could change during the course of the experiment. Therefore, a curve resolution method can be applied to interpret the IMS data. The curve resolution method that is utilized in this work is SIMPLISMA (SIMPLE-to-use Interactive Self-modeling Mixture Analysis), developed by Windig and Guilment. It is both an effective and efficient algorithm for the interpretation of multicomponent mixtures.

The SIMPLISMA algorithm locates pure variables (PVs) in the data set. The PV intensities are used to estimate the concentration profiles of the analytes. There are two requirements for a PV. A PV must be a point in the spectrum that is selective in that it varies correspondingly with a component in a mixture. A PV should not vary with changes in concentration of the other mixture components. The drift time points are evaluated to find the points that have the largest purity and are used to model the concentration profiles. For SIMPLISMA to work, the components of the mixture must vary in concentration during the measurement period.

Determination of the correct number of components or PVs is accomplished by visually examining the pure variables, extracted spectra, and concentration profiles. SIMPLISMA can be performed on a personal computer and is a very rapid calculation
(e.g. less than a minute). Initially, an excessive number of components are arbitrarily selected so that the SIMPLISMA model overfits the data. The extracted spectra and concentration profiles are then examined. Overfitting produces components that model noise, small shifts in peaks, or peak shape variations. Components that characterize noise are eliminated from the SIMPLISMA model, and a reduced number of components are selected. SIMPLISMA is run again with the reduced number of components to furnish accurate concentration profiles and extracted spectra.

Windig and Guilment developed the SIMPLISMA method, and Harrington et al. demonstrated SIMPLISMA of IMS data simplifies the intricacies of the APCI processes and overcomes the peak capacity limits of IMS. The first step of SIMPLISMA is to compute the purity of each drift time for a set of data. This purity is a measure of the relative variations of the intensity values for the drift time multiplied by a measure of independence of the relative variance. Because all the intensities of an IMS peak are correlated with respect to measurement time, the purity also must include a measure of independence for each drift time point. Two types of variations can occur. Variations in intensity that occur at different rates during the experiment are modeled as different components of a mixture. Variations in intensity that are correlated with respect to the measurement time are modeled as the same component.

The intensities at the drift times that furnish the largest purity values are used to model the concentration profiles of each component. The data matrix is regressed onto the concentration profiles using ordinary least squares regression to acquire mathematically extracted spectra. Each spectrum is normalized by the square root of the
sum of squares so that the SIMPLISMA spectral intensities have no units and the vector length of the spectrum is unity. Normalization corrects for scale variation caused by different concentrations and access to a concentration independent spectrum is a valuable asset to IMS. A second regression of the data onto the normalized spectra is used to furnish concentration profiles that have units of intensity.

A 1 µL sample of cocaine at a concentration of 1.0 mg/mL in methanol was placed on an IMS filter. The filter was then placed in a cartridge and analyzed by the spectrometer. Figure 1.6 gives the three-dimensional (3D) surface plot of the results obtained from this experiment. SIMPLISMA factors the data matrix into two matrices that can be viewed with two simple two-dimensional (2D) plots instead of using a 3D plot. The first matrix represents the change in concentration of mixture components with respect to measurement time and is graphed as a function of integrated ion intensity with respect to spectrum number. Figure 1.7 gives the SIMPLISMA concentration profiles for the positive ion mode cocaine experiment. The ordinate corresponds to the integrated intensity of the peaks in units of volts, and the abscissa corresponds to the spectrum scan number.

The concentration of nicotinamide in the spectrometer is high. When the cocaine vapors are carried into the reaction region of the spectrometer, charge transfer occurs. As the nicotinamide ions transfers protons to the cocaine, the number of nicotinamide ions decreases as the cocaine ions form. At the end of the experiment, the concentration of the nicotinamide ions returns to normal, as the cocaine vapors are swept out of the reaction region.
Figure 1.6 Surface plot of a sample of 1 µL of cocaine at a concentration of 1.0 mg/mL in methanol analyzed in positive ion mode on the Barringer Ionscan® 350.
Figure 1.7 SIMPLISMA concentration profiles of 1 µL of cocaine at a concentration of 1.0 mg/mL in methanol analyzed in positive ion mode on the Barringer Ionscan® 350. Components are ordered by SIMPLISMA purity value.
The concentration profiles indicate relative concentrations and do not convey true concentration units. Each component of the concentration profiles then represents a set of scaling factors as a function of spectrum number. For a given spectrum, these scaling factors can be applied to the corresponding extracted spectra.

The sum of the scaled spectra estimates the spectrum in the data at the given spectrum number and produces the second 2D plot. Figure 1.8 gives the SIMPLISMA extracted spectra of a positive ion mode cocaine experiment. Peaks in a SIMPLISMA extracted spectra are grouped according to the rate of growth and decomposition for the peaks. The ordinate corresponds to the relative intensities of the peaks while the abscissa corresponds to the drift times of the peaks in milliseconds. The peaks still exhibit the same drift time and resulting reduced mobilities, so identification is possible. The SIMPLISMA extracted spectra have relative intensity units, so that the absolute intensity unit will be represented by the concentration profile. The data matrix is regressed onto the normalized spectra to furnish concentration profiles that have the same units as the instrument readout (i.e., V).

The SIMPLISMA method is usually applied to a data set of spectra where the components will vary during the course of the measurement; however, SIMPLISMA can also be applied to spectra collected from several different experiments that have been combined into a single data set. SIMPLISMA assumes that the analyte concentration varies independently of the background matrix. This assumption may or may not be true. If the assumption is incorrect, then some of the extracted components may contain features that are from both analyte and background signals.
Figure 1.8 SIMPLISMA extracted spectra of 1 μL of cocaine at a concentration of 1.0 mg/ml in methanol analyzed in positive ion mode on the Barringer Ionscan® 350. Components are ordered by SIMPLISMA purity value.
Major benefits of SIMPLISMA are computational efficiency and simplicity towards the assumptions regarding the concentration profiles. Windig has demonstrated the use of SIMPLISMA with spectral data files including Raman spectra of a reaction followed in time, Fourier transform infrared microscopy spectra of a polymer laminate, near infrared spectra of mixtures of five solvents, and time resolved mass spectra of a three component mixture. SIMPLISMA has also been successfully applied to the deconvolution of multiresponse fluorescence spectra, peak purity assays of multicomponent mixtures by high performance liquid chromatography with diode-array detection, analysis of metal oxide catalysts by diffuse reflectance spectroscopy, and the influence of temperature perturbations on near infrared spectra. SIMPLISMA has been applied to IMS data, and Harrington et al. have demonstrated that SIMPLISMA works well at characterizing multicomponent IMS data.
Chapter 2. Temperature Ramped Thermal Desorption Ion Mobility Spectrometry

Ion mobility spectrometry is routinely used in airports across the nation to screen individuals and luggage for explosives or explosive residues. Ion mobility spectrometry is also used to screen samples at customs and crime scenes for illicit substances, such as methamphetamine, cocaine, heroin, or marijuana. Sampling procedures employed in these circumstances lead to the possibility of compounds entering the spectrometer that could interfere with the analyte of interest. An example of the problem with interferents hindering the detection of analytes of interest would be the problem the Drug Enforcement Agency has encountered with the detection of methamphetamine in the presence of nicotine. The ability to differentiate the drug from background interferences is imperative in prosecuting the suspect. Cigarette smoke residue prevails at clandestine drug laboratories and can interfere with the detection of methamphetamine by generating false negative or false positive responses. These inaccurate responses can be the result of several different factors. A false negative response would occur when the instrument does not alarm, but two unresolved peaks were present or the peak saturated the spectrum. These situations usually involved a small shift in drift time. This can be a potentially serious problem for IMS operators who only rely on the alarm as opposed to a personal computer for interpretation of results. A false positive response would occur when the instruments alarms, but the alarm was due to the presence of nicotine.

Problems like the one previously stated support the use of a separation device before the IMS detector in instances where molecules of similar ionization properties...
may exist in a sample. The distribution of ions in mixed systems is governed by several factors including the charge affinities of the analytes and reactant ions, the concentrations of the analytes, and the conditions of the carrier gas (e.g. temperature and humidity). Instrumental variations can also affect the charge transfer chemistry. One method used to reduce the problems with the response caused by charge transfer reactions is to use GC-IMS. Unfortunately, the advantages of utilizing GC-IMS are offset by several disadvantages including cost, analysis time, and difficulty with nonvolatile, thermally labile, and polar substances.

An alternative method was demonstrated that coupled temperature programming with chemometric processing on IMS data. Temperature adds an extra dimension to the data that aids in the interpretation of complex drug spectra by allowing identification and resolution of analytes in mixtures. The dynamic response of the IMS spectra with respect to desorber temperature may be exploited to resolve target analytes from interfering compounds. The dynamic response is related to vapor pressure and decomposition temperature of the analytes. Therefore, this technique improved the selectivity of the IMS data and allowed for the detection of methamphetamine in the presence of cigarette tar. However, this experiment used an open system, so the results were subject to variations in room temperature, humidity, and pressure. The experiment was also very time-consuming, taking several minutes, so a quicker and more precise method of temperature control was desired.

False negative responses in drug detection are not critical. However, failure to detect explosives may endanger the lives of hundreds of people, such as passengers on an
Recent world events have increased the importance of accurate and rapid screening methods for the detection of explosives and explosive residues in airports. Therefore, the Federal Aviation Administration (FAA) has placed stringent demands upon explosive detection systems. High reliability explosive detection systems must have false positive rates below 5% and even lower false negative rates. The detection system must be sensitive enough to detect less than two pounds of explosives, and the analysis can take no longer than six seconds. Currently, ion mobility spectrometers are utilized in airports across the nation at security checkpoints for the detection of explosives on carry-on luggage including handbags and briefcases. The detection of relatively volatile explosives, like TNT, on luggage may be done by directly sampling the object. However, detection of plastic explosives, like cyclotrimethylene trinitramine (RDX), pentaerythritol tetranitrate (PETN), or cyclotetramethylene tetranitramine (HMX), which have low room temperature vapor pressures require preconcentration of the vapors. Structures for the explosives investigated in this work are given in Figure 2.1, and information regarding the explosives used in these experiments are given in Table 2.1, including molecular mass, experimental reduced mobilities, and reduced mobilities provided by Barringer Instruments Incorporated. Preconcentration is accomplished by trapping microparticles containing the adsorbed explosives vapors on filters. The filters are then transported to the detector where they are heated and analyzed. Because the filters are swiped over the surface being monitored, other substances can be collected on the filter along with the explosive. During analysis, it is possible for these background substances to interfere with the analysis of the explosive, producing inaccurate responses. Because security
Figure 2.1 Structures for (A) 2,4,6-trinitrotoluene (TNT), (B) cyclotrimethylene trinitramine (RDX), (C) pentaerythritol tetranitrate (PETN), and (D) cyclotetramethylene tetranitramine (HMX).
Table 2.1  Explosives investigated with temperature ramped thermal desorption with their molecular masses, peak identifications, experimental reduced mobilities, and reduced mobilities provided by Barringer Instruments Incorporated.\textsuperscript{37} Precision of these reduced mobilities is based on the change in the reduced mobility of the methyl salicylate peak which was $1.56\pm0.03$ cm$^2$V$^{-1}$s$^{-1}$ with the error reported as the 95% confidence limit. This was determined by taking a total of five samples over the course of the entire project.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Mass (g/mol)</th>
<th>Peak Identification</th>
<th>Experimental $K_0$ (cm$^2$V$^{-1}$s$^{-1}$)</th>
<th>Barringer Instruments $K_0$ (cm$^2$V$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNT</td>
<td>227.15</td>
<td>TNT</td>
<td>1.45</td>
<td>1.45</td>
</tr>
<tr>
<td>PETN</td>
<td>316.2</td>
<td>PETN-H</td>
<td>1.23</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PETN-Cl</td>
<td>1.15</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PETN-NO$_3$</td>
<td>1.08</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PETN-F</td>
<td>1.03</td>
<td>1.03</td>
</tr>
<tr>
<td>HMX</td>
<td>296.16</td>
<td>HMX-H</td>
<td>NA</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HMX-Cl</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HMX-NO$_3$</td>
<td>NA</td>
<td>1.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2HMX-Cl</td>
<td>NA</td>
<td>0.82</td>
</tr>
</tbody>
</table>

NA indicates the absence of a peak; therefore, reduced mobilities could not be calculated.
officers only rely on the LCD display on the detector and an alarm, samples could be incorrectly identified.

### 2.1. Experimental Data for Temperature Ramped Desorption

The ion mobility spectrometer used in this work was a Barringer Ionscan® Model 350 (Barringer Instruments Inc., New Jersey, USA). All spectra were acquired in explosives, or negative ion, mode. Methyl salicylate was the internal calibrant, and a structure of methyl salicylate is provided in Figure 2.2. A sample of 1 µL of TNT (6.0 mg/L in deionized water) was run each day experiments were conducted in negative ion mode to calibrate the instrument. Each spectrum comprised 1800 data points. The data acquisition frequency was 80 kHz. Data were collected on a National Instruments™ AT-MIO-16XE-10 board located in a Pentium Pro 200 MHz computer, and the acquisition system was a Laboratory Virtual Instrument Engineering Workbench (LabVIEW) 5.1 virtual instrument built by Guoxiang Chen. Spectra were collected after a 5.0 millisecond delay with a shutter grid width of 200 µs. The scan period was set at 25 milliseconds. The drift flow was 351 cm³/min with a 300 cm³/min-sample flow. This resulted in an exhaust flow of 651 cm³/min for the spectrometer.

The inlet temperature and drift tube temperature remained constant throughout these experiments at 241 and 91 °C, respectively. The desorber heater is usually held constant at 234 °C for the detection of explosives, which results in an isothermal run. However, for this work, the heater was modified and controlled by an external power supply (Tenma™ 72-630 DC power supply). By modifying the desorber heater, the temperature could be precisely controlled and changed during the course of a
Figure 2.2 Structure of negative ion mode calibrant, methyl salicylate.
measurement. The temperature changes were monitored by a homebuilt LabVIEW 5.1 virtual instrument.

Standard solutions were made of all explosives to determine experimental reduced mobilities. These solutions were all made by weighing 6.0 mg of each explosive and dissolving the solid in 1.0 L of deionized water. The solution was stirred by a magnetic stir bar for three hours. To determine the experimental reduced mobilities, 1 µL of an explosive solution was placed on an IMS filter disk. The sample was then analyzed by the Barringer Ionscan® 350. Resulting peaks were used to determine the experimental reduced mobilities, and these reduced mobilities are included in Table 2.1. Precision of these reduced mobilities is based on the change in the reduced mobility of the methyl salicylate peak. The methyl salicylate reduced mobility for this project was 1.56±0.03 cm²V⁻¹s⁻¹ with the error reported as the 95% confidence limit. This was determined by taking a total of five samples over the course of the entire project. Differences between experimental reduced mobilities and Barringer Instruments reduced mobilities could be due to different operating parameters or the purity of the samples investigated. However, experimental reduced mobilities were calculated by using the samples that were used throughout the rest of the experiments, and matches indicated a positive identification.

The 3,5-dichlorophenol and 2,3-dichlorophenol were both purchased from Aldrich Chemical Company, Lot No. 09615KG and 09626CQ, respectively. These two compounds were used as the interferents for this work. They were chosen because most interferents have higher volatilities than the explosives under investigation. A possible interferent in an airport setting would be a perfume or cologne. Standard solutions were
made of the 3,5-dichlorophenol and 2,3-dichlorophenol using the same method described for dissolving the explosives at the same concentration. The experimental reduced mobilities for 3,5-dichlorophenol and 2,3-dichlorophenol were 1.06 and 1.11 cm²V⁻¹s⁻¹, respectively. These experimental values including experimental reduced mobilities for the explosives were used to identify peaks during the course of these experiments. Fiberglass IMS sample filters (Barringer Instruments), Part No. PL09806, were used to collect samples for insertion into the IMS.

Different amounts of PETN, HMX, 3,5-dichlorophenol, and 2,3-dichlorophenol were placed on a tabletop and allowed to air dry at room temperature. For the concentrated samples, 1 µL of the 6.0 mg/L solutions, approximately 6 ng of PETN, HMX, or the corresponding interferent, were placed on the surface. When dilute concentrations were used, approximately 600 pg of PETN, HMX, or the corresponding interferent were placed on the surface. This was done by diluting the original solutions down to 0.6 mg/L. Then, 1 µL of the 0.6 mg/L solution was placed on the surface. The solutions were allowed to air dry, and then the surface was wiped with the IMS sample filter. The filter was then placed in a sample cartridge. The filter was heated at either a constant temperature for the isothermal runs or at changing temperatures for the temperature ramp runs. When the system was isothermally heated, the desorber temperature was set at 234 °C. For the temperature ramped system, the initial temperature was 160 °C and ramped at a rate of 2.0 °C/s. Twenty second runs were necessary for the temperature ramped systems, while 5 second runs were used for the isothermal experiments.
2.2. **Results of Explosive Temperature Ramped Desorption Experiments**

A single IMS spectrum often exhibits a noisy baseline, and peaks can be difficult to identify. Figure 2.3 gives a negative ion spectrum of 1 µL of PETN, at a concentration of 6.0 mg/L in deionized water, at scan number 80. This scan occurred approximately 2 seconds into the isothermal analysis at 234 °C. The ordinate corresponds to the intensity of the peaks in units of volts, while the abscissa corresponds to the drift time of the peak. The drift time of the peak is then used to determine the reduced mobility of the peak. Depending on the temperature of desorption, when heated, the PETN will produce as many as four peaks, and the assignment of these peaks has been made by mass spectrometry. The four peaks are visible in Figure 2.3. The peak at 16.1 ms corresponds to PETN-H, a proton abstraction, with an experimental reduced mobility of 1.23 cm²V⁻¹s⁻¹. The three peaks at 17.4 ms, 18.5 ms, and 19.2 ms correspond to the chloride adduct, nitrate adduct, and a fingerprint oil adduct of PETN with experimental reduced mobilities of 1.15, 1.08, and 1.03 cm²V⁻¹s⁻¹, respectively. In traditional GC-MS detection methods, the decomposition of PETN can make the identification of PETN difficult. By IMS, the production of four peaks from adduct formation can be helpful. In the presence of an interferent, unless all four peaks were affected, PETN could still be identified. An IMS spectrum of 1 µL of an interferent, 3,5-dichlorophenol, at a concentration of 6.0 mg/L in deionized water, at scan number 66, is given in Figure 2.4. This scan occurred approximately 1.6 seconds into the isothermal analysis at 234 °C. The peak appears at a drift time of approximately 18.9 ms and has a corresponding reduced mobility of 1.06 cm²V⁻¹s⁻¹.
Figure 2.3  Negative ion spectrum of a 1 µL sample of PETN at a concentration of 6.0 mg/L in deionized water at scan number 80. This scan occurred approximately 2 seconds into the analysis, and the analysis was conducted isothermally at 234 ºC. This is an example of a standard analysis used to determine experimental reduced mobilities.
Figure 2.4 Negative ion spectrum of a 1 µL sample of 3,5-dichlorophenol at a concentration of 6.0 mg/L in deionized water at scan number 66. This scan occurred approximately 1.6 seconds into the analysis, and the analysis was conducted isothermally at 234 ºC. This is an example of a standard analysis used to determine experimental reduced mobilities.
Figure 2.5 gives the concentration profiles provided by SIMPLISMA of the 1 µL sample of PETN at a concentration of 6.0 mg/L in deionized water during an isothermal analysis. The ordinate corresponds to the intensity of the peaks in units of volts, and the abscissa corresponds to the spectrum scan number or spectrum acquisition time. At the beginning of the experiment, before the PETN sample has been introduced into the IMS, the calibrant and superoxide peaks have large intensities. For simplicity, these two peaks will be jointly referred to as the reactant ion peak (RIP). When the PETN is introduced, the intensity of the RIP decreased as the intensity of the PETN peaks increased. This is typical of IMS data because the intensity of the calibrant RIP varies inversely with respect to the analyte peaks. As the analyte peak increases with concentration, the RIP decreases because of the conservation of charge and competitive charge transfer among ions. At the end of an experiment, the RIP increases to its original intensity as the analyte peaks disappear as the PETN is purged from the reaction region.

Each component represents a set of scaling factors as a function of scan number. For a given spectrum or scan number, these scaling factors can be applied to the corresponding extracted spectra. The sum of the scaled spectra estimates the spectrum in the data at the given scan number. The usefulness of SIMPLISMA can be seen by comparing the SIMPLISMA extracted spectra of a sample of 1 µL of PETN at a concentration of 6.0 mg/L in deionized water during an isothermal run of Figure 2.6 with the raw spectra of Figure 2.3. For Figure 2.6, the ordinate corresponds to a relative intensity of the peak measured in units of volts, while the abscissa corresponds to the drift time of the peaks in milliseconds. These two figures can be compared because the
Figure 2.5 SIMPLISMA extracted concentration profiles for an analysis of 1 µL of PETN at a concentration of 6.0 mg/L in deionized water. This analysis was conducted isothermally at 234 ºC. Components are ordered by SIMPLISMA purity value.
**Figure 2.6** SIMPLISMA extracted negative ion spectra from a 1 µL sample of PETN at a concentration of 6.0 mg/L in deionized water. This analysis was conducted isothermally at 234 ºC. Components are ordered by SIMPLISMA purity value.
abscissas of the figures are identically correlated to each other. The components in the SIMPLISMA extracted spectra are ordered by purity or importance. The peaks still exhibit the same reduced mobilities, so identification is possible. The advantages of using SIMPLISMA include a smoother baseline, improved signal to noise ratio, and the exploitation of temporal information to resolve overlapping peaks.

When PETN and 3,5-dichlorophenol were both placed on the filter for analysis by IMS by wiping the tabletop surface with the IMS filter, one of two problems would occur. If the concentration of PETN was approximately 600 pg and the concentration of the interferent was approximately the same, the interferent, having a greater electron affinity, will completely suppress the signal from the PETN. This behavior is apparent in Figure 2.7. If, in contrast, the concentration of PETN was approximately 6 ng and the concentration of the interferent was approximately 600 pg, the SIMPLISMA extracted spectra provided a cluster of unresolved peaks as given in Figure 2.8. This situation caused an instrumental alarm; however, the amount of PETN necessary to provide this result was high. These two situations demonstrate the problem of compounds interfering with the analytes of interest, in this case, explosives.

When the temperature program was employed, for a mixture of PETN and 3,5-dichlorophenol, the two compounds were separated. The interferent has a higher vapor pressure than the PETN. Therefore, the temperature program was started at a temperature just below the temperature needed to vaporize the PETN. Several different starting temperatures were employed, but it was discovered that starting at 160 °C was sufficient to separate the compounds and produce all of the necessary peaks to identify
Figure 2.7 SIMPLISMA extracted negative ion spectra from a mixture of approximately 600 pg of both 3,5-dichlorophenol and PETN. The absence of PETN peaks means that the PETN has been completely suppressed by the 3,5-dichlorophenol. Components are ordered by SIMPLISMA purity value.
Figure 2.8 SIMPLISMA extracted negative ion spectra from a mixture of approximately 600 pg of 3,5-dichlorophenol and approximately 6 ng of PETN. The spectra shows a cluster of unresolved peaks. Components are ordered by SIMPLISMA purity value.
the analytes. The temperature was then ramped at a rate of 2.0 °C/s. This temperature ramp was chosen for two specific reasons. To precisely control the temperature, the capabilities of the heater had to be taken into account. The desorber heater could not accurately ramp at a faster rate than 4.0 °C/s. The rate of 2.0 °C/s produced the best separation of the two compounds. This rate allowed the interferent to reach the reaction region and enter the drift region before the PETN was vaporized from the filter. Therefore, when the desorber heater reached a temperature to vaporize the PETN, the interferent was no longer present. An alarm from the instrument resulted. Figure 2.9 gives a three-dimensional plot of a mixture of PETN and 3,5-dichlorophenol with the temperature ramp at approximate concentrations of 600 pg. Figure 2.10 gives the average IMS spectrum of the experiment with the temperature ramp. As discussed earlier, SIMPLISMA allows one to view the three-dimensional data in two easier to interpret two-dimensional plots and allows temporal information to be taken into account that is lost in the average spectrum of the experiment. Figure 2.11 and Figure 2.12 give the two resulting SIMPLISMA figures. Figure 2.11 gives the SIMPLISMA extracted spectra. The concentration of PETN and the 3,5-dichlorophenol for this figure was approximately 600 pg, but when the temperature ramp was employed, the four PETN peaks could be resolved from the interferent peak. Figure 2.12 gives the SIMPLISMA extracted concentration profiles with the temperature ramp. At the beginning of the experiment, the RIP decreases until it is depleted. The beginning temperature was set at 160 °C. As the temperature ramp began, the interferent peak grew as the RIP diminished. As the temperature changed, the interferent peak began to disappear which indicates that the
Figure 2.9 Surface plot of a mixture of approximately 600 pg of both PETN and 3,5-dichlorophenol with a temperature ramp applied to separate compounds.
Figure 2.10  Average ion mobility spectrum of a mixture of approximately 600 pg of both 3,5-dichlorophenol and PETN with temperature ramped thermal desorption applied during data collection. The PETN-Cl and 3,5-dichlorophenol peaks are identified.
Figure 2.11 SIMPLISMA extracted spectra of a mixture of approximately 600 pg of both 3,5-dichlorophenol and PETN with temperature ramped thermal desorption applied during data collection. SIMPLISMA exploits temporal changes and shows the four PETN peaks resolved from the 3,5-dichlorophenol peak. Components are ordered by SIMPLISMA purity value.
Figure 2.12 SIMPLISMA extracted concentration profiles for mixture of approximately 600 pg of both 3,5-dichlorophenol and PETN with a temperature ramp applied during data collection. These profiles show the differences in growth and decay of peaks between the 3,5-dichlorophenol and the PETN resulting when the temperature ramp was applied. Components are ordered by SIMPLISMA purity value.
interferent was volatilized and removed from the filter. As the temperature increased, the PETN peak grows indicating that the necessary temperature was being reached to vaporize the PETN.

A second explosive was investigated in this work to ensure that reproducible results were obtainable. The HMX utilized in this experiment has two consistent peaks at drift times of approximately 16.9 and 19.2 ms, which correspond to reduced mobilities of 1.25 and 1.09 cm$^2$V$^{-1}$s$^{-1}$, respectively. The peak at 17.0 ms corresponds to the chloride adduct for HMX,\textsuperscript{37,39} and the peak at 19.3 ms has not been identified. The interferent tested with HMX was 2,3-dichlorophenol. The approximate drift time of the interferent was 19.0 ms with a reduced mobility of 1.11 cm$^2$V$^{-1}$s$^{-1}$. When the two compounds were placed on the IMS filter for analysis by swiping the tabletop surface, the interferent completely suppressed the HMX even with 6 ng of HMX on the disk. However, when the temperature ramp of the desorber heater was applied, the HMX was no longer suppressed by the interferent, and the HMX and interferent peaks were resolved. The instrument alarmed for the presence of HMX. Figure 2.13 gives an average IMS spectrum of the temperature ramp experiment. By using SIMPLISMA, the separation between HMX and 2,3-dichlorophenol can be easily visualized in Figure 2.14 and important temporal information is gained by Figure 2.15.

2.3. Discussion and Conclusions of Temperature Ramped Desorption

Coupling a temperature ramped thermal desorption with IMS produced a system with greater sensitivity and enhanced selectivity. SIMPLISMA allows the detection of IMS features that would normally be missed by visual examination of IMS spectra.
Figure 2.13 Average ion mobility spectrum of a mixture of approximately 600 pg of both 2,3-dichlorophenol and HMX with temperature ramped thermal desorption applied during data collection. The HMX-Cl and 2,3-dichlorophenol peaks are identified.
Figure 2.14 SIMPLISMA extracted spectra of a mixture of approximately 600 pg of both 2,3-dichlorophenol and HMX with temperature ramped thermal desorption applied during data collection. SIMPLISMA exploits temporal changes and shows the HMX peaks resolved from the 2,3-dichlorophenol peak. Components are ordered by SIMPLISMA purity value.
Figure 2.15 SIMPLISMA extracted concentration profiles for mixture of approximately 600 pg of both 2,3-dichlorophenol and HMX with a temperature ramp applied during data collection. These profiles show the differences in growth and decay of peaks between the 2,3-dichlorophenol and the HMX resulting when the temperature ramp was applied. Components are ordered by SIMPLISMA purity value.
Because SIMPLISMA takes advantage of changes that occur in the spectral features with respect to time, the method is well suited for instrumentation that uses temperature programs for sample volatilization and decomposition. The concentration profiles provide valuable information regarding the changes in extracted spectral features with time or temperature. Substances of similar size and charge can be separated based on their temperature of vaporization. Volatility adds an extra dimension to the analysis, allowing the resolution of mixture components. Also, the complementary nature of the spectral and concentration profiles allows trends in the entire data set to be visually assessed. Unfortunately, the temperature ramp increased the length of analysis to 20 seconds whereas a traditional analysis would take approximately 5 seconds.
Chapter 3. Solid Phase Extraction Ion Mobility Spectrometry

Traditionally, liquid-liquid extractions were used for sample preparation, which consisted of sample dissolution, purification, and extraction. The disadvantages with liquid-liquid extractions include the use of large volumes of organic solvent, cost, possible creation of emulsions, and exposure to potentially hazardous solvents. Solid phase extraction (SPE) is a method of sample preparation that concentrates and purifies analytes from solution by sorption onto a solid-phase cartridge or disk. Removal of the analyte from the cartridge or disk usually involves elution with a solvent appropriate for the type of instrumental analysis to be employed. The mechanisms of retention include reversed phase, normal phase, and ion exchange. Solid phase extraction replaces liquid-liquid extraction as a sample preparation tool and provides a method that is simple and safe to use. The benefits of SPE include high recoveries of analytes, purified extracts, ease of automation, compatibility with chromatographic analysis, and reduction in the consumption of organic solvents.49

Because SPE disks were employed in this work, the process used for extraction by disks will be discussed. Solid phase extraction disks were chosen over other methods such as solid phase microextraction because 3M Empore™ SPE disks closely resemble IMS filter disks. Therefore, no modification of the inlet system on the spectrometer was necessary. Two other major advantages of the disk format are rapid mass transfer because of the greater surface area of the particles and reduced channeling effects because of their large cross-sectional area and thin bed. These properties result in high
flow rates for large volume samples, which is especially useful for large volume samples, greater than one liter, and trace analyses.\textsuperscript{50}

SPE is a four-step process. First, the solid-phase sorbent is cleaned and conditioned. A solvent is passed through the sorbent to clean and wet the packing material and to solvate the functional groups of the sorbent. Typically, the cleaning solvent is the eluting solvent and the conditioning solvent is methanol, which is followed with distilled water. After conditioning, it is important that the disk does not dry out or poor recoveries may result. Next, the sample with the analyte of interest should be applied to the disk. A washing step must then follow, which washes interferences from the disk. The analyte is bound to the disk by sorption. The analyte must then be removed from the disk with an appropriate eluting solvent or by other physical means.\textsuperscript{51}

3.1. \textbf{Trace Explosive Detection in Aqueous Samples}

Nitroaromatic and nitramine explosives are present in the soil and groundwater at more than thirty U.S. Army installations in the United States.\textsuperscript{52} Potential contamination of drinking water has led to extensive networks of groundwater-monitoring wells. The Environmental Protection Agency (EPA) has issued health advisories for several common explosives that could contaminate drinking water. Included in this list are HMX, RDX, and TNT. These proposed drinking water limits range from 400 $\mu$g/L for HMX\textsuperscript{53} to 2.0 $\mu$g/L for both RDX\textsuperscript{54} and TNT\textsuperscript{55}. These advisories are necessary because exposure to these explosives presents a human health risk. In 1999, TNT and RDX were ranked as 82 and 85, respectively, on the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) list of priority hazardous substances.\textsuperscript{56}
Overexposure to these compounds can result with serious effects on neurological, hepatic, and reproductive systems, and RDX and TNT have been shown to cause cancer.\textsuperscript{57-60}

Several methods have been developed for the detection of explosives and explosive residues in groundwater and drinking water. The current method utilized by the EPA is SW 846 Method-8330, which uses either salting-out or solid-phase extraction and the analysis of the acetonitrile extract using high-performance liquid chromatography (HPLC) equipped with an ultraviolet (UV) detector for the detection of nitroaromatic and nitramine explosives. However, the response of the UV detector is susceptible to interferences produced by co-eluting compounds.\textsuperscript{61-63} Other measurement procedures include extraction by SPE and detection by gas chromatography electron capture detection (GC-ECD) \textsuperscript{64} or mass spectrometry (GC-MS) \textsuperscript{65}. However, reproducibility suffers especially for thermally labile compounds such as RDX and HMX, which decompose at the high temperatures required to volatilize these samples in the GC inlet. These methods are also inconvenient because HPLC and GC systems are not currently suitable on-site monitoring devices and sample turnaround time is lengthy. Immunoassays have been proposed as an alternative, and fiber optic probes utilizing different fluorescent immunoassays have been used for the detection of TNT and RDX.\textsuperscript{66-68} However, these methods do not allow for the monitoring of all possible explosive contaminants simultaneously.

Ion mobility spectrometry offers the advantages of both real-time and on-site monitoring capabilities. The detection of substances in liquid matrices is possible by IMS.\textsuperscript{69,70} Early studies by Garofolo et al. used IMS for the detection and identification of
trace levels of explosives in environmental samples after SPE and HPLC fractionation. Explosive-containing HPLC fractions were analyzed by IMS after removal of solvent by vacuum centrifugation. Detection limits were an order of magnitude lower than those for UV detection. Atkinson et al. investigated contaminated soil samples by IMS showing that several compounds can be quantified simultaneously in an acetone extract within a few seconds at a cost of under a dollar/sample.

Coupling SPE directly to IMS yields a rapid, inexpensive method to detect low levels of explosives in aqueous samples. Solid phase extraction provides the benefits of selectively concentrating the analytes and sample clean-up. Because thermal desorption is used, no solvents are required for extraction. Solid phase extraction disks incorporate the extraction method currently regulated by the EPA and afford ease of use with the ion mobility spectrometer. Figure 3.1 shows the scheme utilized in the SPE-IMS experiments. The explosives of interest and possible interferents are run through SPE disks (right) which are very similar in size and shape to fiberglass IMS sample filters (left). The explosives selectively adsorb to the disks while interferences flow through the disks into a waste receptacle. The SPE disk is then placed in a sample cartridge, and the sample cartridge is slid into place above the desorber heater and below the IMS detector inlet. The sample is then heated by the desorber heater and analytes desorb from the SPE disk and enter the spectrometer. If explosives are present, the instrument will alarm.

### 3.2. Experimental Data for Explosives

The ion mobility spectrometer used in this work was a Barringer Ionscan® Model 350 (Barringer Instruments Inc., New Jersey, USA). The spectra were acquired in
Figure 3.1 Scheme utilized during SPE-IMS experiments.
negative ion, or explosives, mode. Each spectrum comprised 2000 data points. The data acquisition frequency was 80 kHz. Data were collected on a National Instruments™ AT-MIO-16XE-10 board located in a Pentium Pro 200 MHz computer, and the acquisition system was a homebuilt LabVIEW 5.1 virtual instrument. Spectra were collected after a 5.00-millisecond delay with a shutter grid width of 200 µs. The scan period was set to 25 milliseconds, and desorption time was set at 20 s. The drift flow was 300 cm³/min with a 284 cm³/min-sample flow. This resulted in an exhaust flow of 584 cm³/min for the spectrometer.

The inlet temperature and drift tube temperature remained constant throughout the experiments at 242 and 92 °C, respectively. The desorber heater is usually held constant at 234 °C for the detection of explosives. For this experiment, different desorption temperatures (100°C - 300°C) were applied to determine the optimal temperature needed to extract the explosive from the SPE disks. All measurements used isothermal desorption, and the optimal desorption temperature was found to be 275 °C. This temperature was the highest possible temperature that did not destroy the SPE disk.

Structures for all explosives investigated in this work are given in Figure 2.1. Standard solutions were made of TNT, RDX, and HMX to determine experimental reduced mobilities. These solutions were all made by weighing 0.5 mg of each explosive and dissolving the solid in 1.0 L of deionized water. The solutions were stirred by magnetic stir bars for three hours. To determine experimental reduced mobilities, 1 µL of an explosive solution was placed on an IMS filter disk. The sample was then analyzed by the Barringer Ionscan® 350. Resulting peaks were used to determine the experimental
reduced mobilities, and this information is included in Table 3.1 along with information regarding molecular mass and reduced mobilities provided by Barringer Instruments Incorporated.\textsuperscript{37} Precision of these reduced mobilities is based on the change in the reduced mobility of the methyl salicylate peak. The methyl salicylate peak reduced mobility for this project was 1.61±0.03 cm\(^2\)V\(^{-1}\)s\(^{-1}\) with the error reported as the 95% confidence limit. This was determined by taking five samples over the course of the entire project. By using samples over the course of the project, shifts in the peak could be used to determine changes in the reduced mobilities. Experimental \(K_0\) values were used to identify unknown peaks during the course of the experiments.

Differences between experimental reduced mobilities and Barringer Instruments reduced mobilities could be due to different operating parameters or the purity of the samples investigated. However, experimental reduced mobilities were calculated by using the samples that were used throughout the rest of the experiments, and \(K_0\) matches indicated a positive identification.

Standard solutions of the explosives were also prepared in drinking water and Hocking River (Athens, OH) water using the same method of dilution as the standard solutions. When very low concentrations were used, serial dilutions were performed. After each dilution, the solution would be stirred for one hour by a magnetic stir bar.

Fiberglass IMS sample filters (Barringer Instruments), Part No. PL09806, were used to collect samples for insertion into the IMS for standard runs. Commercially available high performance extraction disks by 3M Empore\textsuperscript{TM} were investigated in this work including SDB-RPS (poly(styrenedivinylbenezene) copolymer that has been
Table 3.1 Explosives investigated with SPE-IMS with their molecular masses, peak identifications, experimental reduced mobilities, and reduced mobilities provided by Barringer Instruments Incorporated. Precision of these reduced mobilities is based on the change in the reduced mobility of the methyl salicylate peak which was 1.61±0.03 cm²V⁻¹s⁻¹ with the error reported as the 95% confidence limit. This was determined by taking five samples over the course of the project.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Molecular Mass (g/mol)</th>
<th>Peak Identification</th>
<th>Experimental K₀ (cm²V⁻¹s⁻¹)</th>
<th>Barringer Instruments K₀ (cm²V⁻¹s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNT</td>
<td>227.2</td>
<td>TNT</td>
<td>1.45</td>
<td>1.45</td>
</tr>
<tr>
<td>RDX</td>
<td>222.6</td>
<td>RDX-Cl</td>
<td>1.39</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RDX-NO₃</td>
<td>1.32</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RDX-F</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2RDX-Cl</td>
<td>NA</td>
<td>0.95</td>
</tr>
<tr>
<td>HMX</td>
<td>296.2</td>
<td>HMX-H</td>
<td>1.31</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HMX-Cl</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HMX-NO₃</td>
<td>1.16</td>
<td>1.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2HMX-Cl</td>
<td>NA</td>
<td>0.82</td>
</tr>
</tbody>
</table>

NA indicates the absence of a peak; therefore, reduced mobilities were not calculated.
modified with sulfonic acid groups to make it hydrophilic), C-8 (octyl), and C-18 (octadecyl) disks. The SDB-RPS disks produced the best overall results and were This included larger peaks with the SDB-RPS disks, and the fact that the C-8 and C-18 disks could only be used once. At the temperature necessary to desorb the explosives from the disks, the C-8 and C-18 extraction disks were destroyed. The SDB-RPS disks, however, were not destroyed and could be reused without loss of signal. A comparison of the peak heights by using the different extraction disks can be viewed in Table 3.2.

The procedure utilized to clean and condition the SDB-RPS extraction disk is as follows. A SPE disk was placed in a reservoir and a vacuum was applied to pull the cleaning and conditioning solvents through the disk. First, the disks were cleaned by adding 10 mL of acetone to the reservoir above the disk. After the disk dried, 10 mL of isopropanol was added to the reservoir above the disk. After the disk dried, the disk was conditioned by adding 5 mL of methanol to the reservoir. When the methanol level reached approximately 1 mm above the disk, 10 mL of reagent grade water was added to the reservoir. When the water level reached approximately 1 mm above the disk, 500 mL of explosive spiked sample was run through the SPE disk. Explosive spiked samples were also poured through IMS fiberglass sample filters for comparison. The disk was dried before IMS analysis was conducted.

HPLC grade methanol and ACS certified acetone were both purchased from Fischer Scientific, Lot No. 001927 and 982512, respectively. Isopropanol was purchased from Eastman Kodak Company, Lot No. 714-5.
Table 3.2 Comparison of explosive peak height results with extraction by 3M Empore™ SDB-RPS, C-8, and C-18 solid phase extraction disks. Peak height in units of volts is reported as an average of three runs with the 95% confidence limit.

<table>
<thead>
<tr>
<th>Explosive</th>
<th>High Performance SPE Disk</th>
<th>Peak Height (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNT (0.50 μg/L)</td>
<td>SDB-RPS</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>C-8</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>C-18</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>RDX (0.50 μg/L)</td>
<td>SDB-RPS</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>C-8</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>C-18</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>HMX (0.50 μg/L)</td>
<td>SDB-RPS</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>C-8</td>
<td>0.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>C-18</td>
<td>0.3 ± 0.2</td>
</tr>
</tbody>
</table>
3.3. Results of Explosive Experiments

Figure 3.2 gives a negative ion spectrum of TNT at a concentration of 0.50 mg/L in deionized water at scan number 200, approximately 5 seconds into the analysis. This experiment was conducted isothermally at 234 °C. In this figure, the chloride ion (Cl⁻) peak is also evident. This peak results from the hexachloroethane that is combined with the sample carrier gas in negative ion mode on the Ionscan® Model 350. The ordinate corresponds to the intensity of the spectrum in volts, while the abscissa corresponds to the drift time measurement. TNT has a characteristic peak at 14.6 ms that corresponds to a $K_0$ of 1.45 cm²V⁻¹s⁻¹. Figure 3.3 gives a 3D representation of a single IMS experiment. At the beginning of a run, the calibrant and superoxide ($O_2^-$) peaks appear at approximately 13.1 and 10.1 ms, respectively. For convenience, these two peaks are collectively termed the RIP. As the run progresses, the RIP disappear, and the chloride ion and TNT peaks appear at approximately 9.8 and 14.6 ms, respectively. At the end of the experiment, the chloride ion and TNT peaks disappear, and the RIP reappear. From this figure, it is not difficult to identify the TNT peak; however, separating the chloride ion peak from the superoxide peak may be difficult because of the similarity in drift times. Also, when more intricate spectra (i.e. compounds that produce several peaks) are collected, the concentration of the analyte under investigation is low, or several peaks change differently with respect to time, interpreting the data with a 3D graph can be very difficult. A single IMS spectrum using the Barringer Ionscan® Model 350 often also exhibits a noisy baseline making smaller peaks difficult to identify.
Figure 3.2 Negative ion spectrum of 1 µL sample of TNT at a concentration of 0.50 mg/L in deionized water at scan number 200. This scan occurred approximately 5 seconds into the analysis, and the analysis was conducted isothermally at 234 ºC. This is an example of a standard analysis used to determine reduced mobilities.
Figure 3.3 Surface plot of a 1 µL sample of TNT at a concentration of 0.50 mg/L in deionized water. The analysis was conducted isothermally at 234 ºC.
Figure 3.4 and Figure 3.5 give the SIMPLISMA extracted spectra and concentration profiles obtained from the same experiment represented in Figure 3.2 and Figure 3.3. Figure 3.4 provides the extracted spectra for the run, and models correlated peaks together, in that peaks that grow and decay at the same rate are modeled in the same component. The ordinate corresponds to the relative intensities of the peaks while the abscissa corresponds to the drift times of the peaks in milliseconds. The rates are then represented in Figure 3.5 by the concentration profile. The ordinate corresponds to the intensity of the peaks in units of volts, and the abscissa corresponds to the spectrum scan number.

Figure 3.6 and Figure 3.7 give negative ion spectra of 1 µL samples of RDX and HMX in deionized water at concentrations of 0.50 mg/L at scan numbers 200, respectively. These scans occur approximately 5 seconds into the analyses. Figure 3.8 and Figure 3.9 give the extracted spectra for RDX and HMX, respectively. Figure 3.10 gives the average negative ion spectrum of a 1 µL sample of RDX at a concentration of 0.50 mg/L in deionized water. By comparing Figure 3.8 with Figure 3.10, one advantage of SIMPLISMA becomes apparent. SIMPLISMA has the ability to extract peaks that could otherwise be unnoticed. RDX can produce as many as four peaks when under investigation by thermal desorption IMS by the Barringer Ionscan® Model 350; however, it should be noted that the presence of any RDX peak warrants further investigation and should indicate the existence of RDX in the sample.\textsuperscript{37,39} Several factors influence the presence of peaks. The presence of the chloride adduct peak is dependent on the chloride concentration in the sample flow gas. The nitrate adduct is determined by the amount of
Figure 3.4 SIMPLISMA extracted negative ion spectra from a 1 µL sample of TNT at a concentration of 0.50 mg/L in deionized water. This analysis was conducted isothermally at 234 °C. Components are ordered by SIMPLISMA purity value.
Figure 3.5 SIMPLISMA extracted concentration profiles for an analysis of 1 µL of TNT at a concentration of 0.50 mg/L in deionized water. This analysis was conducted isothermally at 234 ºC. Components are ordered by SIMPLISMA purity value.
Figure 3.6 Negative ion spectrum of a 1 µL sample of RDX at a concentration of 0.50 mg/L in deionized water at spectrum number 200. This scan occurred approximately 2 seconds into the analysis, and the analysis was conducted isothermally at 234 °C. This is an example of a standard analysis used to determine experimental reduced mobilities.
Figure 3.7 Negative ion spectrum of a 1 µL sample of HMX at a concentration of 0.50 mg/L in deionized water at spectrum number 200. This scan occurred approximately 2 seconds into the analysis, and the analysis was conducted isothermally at 234 ºC. This is an example of a standard analysis used to determine experimental reduced mobilities.
Figure 3.8 SIMPLISMA extracted negative ion spectra from a 1 µL sample of RDX at a concentration of 0.50 mg/L in deionized water. This analysis was conducted isothermally at 234 ºC. Components are ordered by SIMPLISMA purity value.
Figure 3.9 SIMPLISMA extracted negative ion spectra from a 1 µL sample of HMX at a concentration of 0.50 mg/L in deionized water. This analysis was conducted isothermally at 234 ºC. Components are ordered by SIMPLISMA purity value.
Figure 3.10 Average negative ion mobility spectrum of a 1 µL sample RDX at a concentration of 0.50 mg/L in deionized water. This analysis was conducted isothermally at 234 ºC. This figure shows that averaging can make peak identification difficult.
nitrate available. The fingerprint oil adduct is only possible if the sample is handled by hand without gloves, and the dimer for any explosive is only possible at very high concentrations of the explosive.\textsuperscript{60}

Three of the four peaks for RDX are clearly visible in Figure 3.8. The four peaks that are possible for RDX include the chloride adduct, the nitrate adduct, the fingerprint oil adduct, and RDX dimer-chloride adduct, and these peaks have been identified by mass spectrometry.\textsuperscript{37, 39} The peak at 15.3 ms, or $K_0$ of 1.39 cm$^2$V$^{-1}$s$^{-1}$, corresponds to the RDX-Cl peak, which is a chloride adduct. The RDX-N$O_3$ peak, a nitrate adduct, is found at a drift time of 16.1 ms with a corresponding $K_0$ of 1.31 cm$^2$V$^{-1}$s$^{-1}$. The third peak, RDX-F, is a fingerprint oil adduct, and the peak appears at a drift time and $K_0$ of 17.5 ms and 1.20 cm$^2$V$^{-1}$s$^{-1}$, respectively. The RDX dimer-chloride adduct peak is not present in this sample. The 2RDX-Cl peak corresponds to a RDX dimer with a chloride adduct. The concentration of the RDX in this run was not high enough for dimer formation. In the average spectrum, Figure 3.10, the peaks are much more difficult to identify, and the possibility of not identifying the RDX-F peak is possible.

HMX has three consistent peaks that can be seen in Figure 3.9. These peaks appear at drift times of approximately 16.3, 17.2, and 18.4 ms that correspond to reduced mobilities of 1.31, 1.24 and 1.16 cm$^2$V$^{-1}$s$^{-1}$, respectively. The peak at 16.3 ms corresponds to an ion formed by proton abstraction of HMX and is called HMX-H. The peak at 17.2 ms corresponds to the HMX-Cl, the chloride adduct of HMX. The peak at 18.4 ms is HMX-NO$_3$, the nitrate adduct. It is marginally different from the Barringer Instrument value of a $K_0$ of 1.19 cm$^2$V$^{-1}$s$^{-1}$. Once again there is the possibility of forming
an HMX dimer-chloride adduct (2HMX-Cl). However, the concentration in this work was low enough so that the dimer ion was not observed.

For the SPE experiments, standard dilutions of TNT, RDX, and HMX were made in drinking water and in river water obtained from the Hocking River (Athens, OH). The SDB-RPS (styrene divinylbenezene-reverse phase sulfonated) extraction disks were cleaned and conditioned before sample extraction. The disks were cleaned by wetting with several milliliters of acetone and isopropanol and drying between each addition. Then the disks were conditioned by applying 5 mL of methanol and 10 mL of reagent grade water to the reservoir. After conditioning, 500 mL samples were run through the SPE disks. Samples were also poured through IMS fiberglass sample filters for comparison. The disks were dried before placing them above the IMS desorber heater. Peaks appeared for all explosive compounds at lower concentrations with the SPE disks than with the IMS filters.

Figure 3.11 and Figure 3.12 give the results of the experiment run with 500 mL of TNT spiked drinking water at a concentration of 4.0 µg/L using an IMS fiberglass filter. Figure 3.11 gives a raw spectrum, and Figure 3.12 gives the SIMPLISMA extracted spectra for the run. The IMS filter did not extract the TNT from the solution, and the TNT does not appear in these graphs. Figure 3.13 and Figure 3.14 give the results from the same concentration of TNT with SPE. A TNT peak is clearly apparent in these two graphs and is easily identified. A concentration of 4.0 µg/L was investigated because it was below the detection limit of IMS.
Figure 3.11 Negative ion spectrum from a 500 mL sample of 4.0 μg/L TNT run at spectrum number 200 utilizing a fiberglass IMS filter. This analysis was conducted isothermally at 275 ºC. The IMS filter did not extract the TNT from the solution, and no TNT peak is present in the spectrum.
Figure 3.12 SIMPLISMA extracted negative ion spectra from 500 mL of 4.0 µg/L TNT utilizing fiberglass IMS filter. This analysis was conducted isothermally at 275 ºC. The IMS filter did not extract the TNT from the solution, and no TNT peak is present in the spectra. Components are ordered by SIMPLISMA purity value.
Figure 3.13 Negative ion spectrum from a 500 mL sample of 4.0 µg/L TNT at spectrum number 200 after solid phase extraction. This analysis was conducted isothermally at 275 ºC. The SPE disk extracted the TNT from the sample, and when analyzed by IMS, the TNT was desorbed from the SPE disk and resulting in the presence of a TNT peak in the ion mobility spectrum.
Figure 3.14 SIMPLISMA extracted negative ion spectra from a 500 mL sample of 4.0 µg/L TNT after solid phase extraction. This analysis was conducted isothermally at 275 ºC. The SPE disk extracted the TNT from the sample, and when analyzed by IMS, the TNT was desorbed from the SPE disk and resulting in the presence of a TNT peak in the SIMPLISMA extracted spectra. Components are ordered by SIMPLISMA purity value.
Figure 3.15 gives a spectrum of a 500 mL sample of TNT spiked drinking water at a concentration of 0.10 µg/L, which is below the EPA regulation of 2.0 µg/L. The TNT peak is discernable in the spectrum. Figure 3.16 demonstrates the value of SIMPLISMA by improving the signal-to-noise ratio of the TNT peak. The TNT peak is modeled with the chloride ion peak in this experiment because the TNT concentration was low enough that the chloride and TNT ions were correlated in time during this experiment. Figure 3.17 and Figure 3.18 also give SIMPLISMA extracted spectra of 500 mL samples of TNT spiked drinking water at concentrations of 0.10 µg/L. All three of these experiments were conducted on the same day. These figures show the reproducibility of this extraction.

SPE coupled to IMS furnishes lower detection limits for TNT in drinking water. Table 3.3 provides detection limits achieved by combining SPE with IMS. These detection limits were determined by taking three times the standard deviation of the noise. Samples were then run at the detection limit concentration and proven by the detection of the analyte peak in the SIMPLISMA spectra. Figure 3.19 gives a SIMPLISMA extracted spectra of a 500 mL sample of TNT in drinking water at a concentration of 0.001 µg/L. Without the use of SIMPLISMA, the detection limits would not be as low.

Earlier, another advantage of using IMS over some of the other techniques was mentioned. This advantage was the simultaneous determination of several explosives at one time. Figure 3.20 gives a SIMPLISMA extracted spectra of a SPE-IMS run with a 500 mL sample of TNT, RDX, and HMX present in the drinking water at equivalent concentrations of 0.50 µg/L. From this figure, the TNT peak is present at a $K_0$ of 1.45
Figure 3.15 Negative ion spectrum of a 500 mL sample of 0.10 µg/L TNT at scan number 200 after solid phase extraction. This analysis was conducted isothermally at 275 ºC. The SPE disk extracted the TNT from the sample, and when analyzed by IMS, the TNT was desorbed from the SPE disk and resulting in the presence of a TNT peak in the ion mobility spectrum. The concentration of 0.10 µg/L is below the EPA regulation limit of 2.0 µg/L.
Figure 3.16 SIMPLISMA extracted negative ion spectra from a 500 mL sample of 0.10 µg/L TNT after solid phase extraction. This analysis was conducted isothermally at 275 ºC. The SPE disk extracted the TNT from the sample, and when analyzed by IMS, the TNT was desorbed from the SPE disk and resulting in the presence of a TNT peak in the ion mobility spectrum. The concentration of 0.10 µg/L is below the EPA regulation limit of 2.0 µg/L. Components are ordered by SIMPLISMA purity value.
Figure 3.17 SIMPLISMA extracted negative ion spectra from a 500 mL sample of 0.10 µg/L TNT after solid phase extraction. This analysis was conducted isothermally at 275 °C. This figure should be compared to Figure 3.16 and Figure 3.18 to show reproducibility. Components are ordered by SIMPLISMA purity value.
**Figure 3.18** SIMPLISMA extracted negative ion spectra from a 500 mL sample of 0.10 µg/L TNT after solid phase extraction. This analysis was conducted isothermally at 275 °C. This figure should be compared to Figure 3.16 and Figure 3.17 to show reproducibility. Components are ordered by SIMPLISMA purity value.
Table 3.3 Comparison of detection limits without solid phase extraction, detection limits after solid phase extraction, and breakthrough concentrations of explosives with extraction.

<table>
<thead>
<tr>
<th>Explosive</th>
<th>Detection Limit without SPE (µg/L)</th>
<th>Detection Limit after SPE (µg/L)</th>
<th>Breakthrough Concentration (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNT</td>
<td>5.0</td>
<td>0.001</td>
<td>7.5</td>
</tr>
<tr>
<td>RDX</td>
<td>12</td>
<td>0.02</td>
<td>20</td>
</tr>
<tr>
<td>HMX</td>
<td>35</td>
<td>0.1</td>
<td>50</td>
</tr>
</tbody>
</table>
Figure 3.19  SIMPLISMA extracted spectra from a 500 mL sample of 0.001 µg/L TNT after solid phase extraction. This analysis was conducted isothermally at 275 ºC. Components are ordered by SIMPLISMA purity value.
Figure 3.20 SIMPLISMA extracted spectra of a 500 mL sample of a mixture of TNT, RDX, and HMX in drinking water at concentrations of 0.50 µg/L after solid phase extraction. This analysis was conducted isothermally at 275 ºC. The presence of peaks for TNT, RDX, and HMX indicate that the simultaneous detection of these three explosives is possible by SPE-IMS. Components are ordered by SIMPLISMA purity value.
cm$^2$V$^{-1}$s$^{-1}$, the RDX-Cl and RDX-NO$_3$ peaks are present at reduced mobilities of 1.37 and 1.31 cm$^2$V$^{-1}$s$^{-1}$, respectively, and the HMX-Cl peak is present at a $K_0$ of 1.24 cm$^2$V$^{-1}$s$^{-1}$.

The RDX-NO$_3$ peak is small, but still visible from the SIMPLSIMA results. There is a slight possibility that the peak at a $K_0$ of 1.31 cm$^2$V$^{-1}$s$^{-1}$ could also be a HMX-H. I have stated that the peak is a result of RDX-NO$_3$. The evidence of my supporting this belief is as follows. When experiments were conducted with only TNT and HMX present, this peak was not evident. When experiments were conducted with only TNT and RDX present, this peak was evident. Therefore, I believe the peak is a result of RDX-NO$_3$.

The simultaneous detection and identification of these three explosives in water is possible with SPE-IMS. At lower concentrations (i.e. 0.05 µg/L), the RDX-NO$_3$ and HMX-Cl peaks disappear; however, the TNT and RDX-Cl peaks are still detectable. These peaks disappear when concentrations below their detection limits are studied.

Two possible problems that could occur were investigated. The first of these involved the desorber heater and thermal decomposition of the SPE disks. When the temperature of desorption was too high, breakdown of the SPE disks occurred and the resulting spectra can be seen in Figure 3.21. The disks, which are cream colored, would turn brown indicating the destruction of the disk. However, if the temperature was not sufficiently high, the explosives would not completely desorb from the SPE disk. An optimal temperature of 275 °C was determined. This temperature was the highest possible temperature that did not destroy the SDB-RPS extraction disk. Multiple runs on the IMS were conducted at this temperature with the same disk to ensure repeatability.
**Figure 3.21** SIMPLISMA extracted spectra of a SDB-RPS extraction disk heated to a desorption temperature of 300 °C. Components are ordered by SIMPLISMA purity values.
Recovery experiments were also investigated at 275 °C, and the results are given in Table 3.4. Because of the problems associated with quantitation in IMS, recovery experiments were conducted by comparing peaks after solid phase extraction for a dilute solution with peaks obtained by placing an equivalent amount of explosive directly on the solid phase disk. An example of how the recovery experiments were conducted follows. A 500 mL sample of TNT spiked drinking water at a concentration of 0.50 µg/L was run through an SDB-RPS extraction disk, and 0.25 µg of TNT should be retained on the extraction disk. Therefore, 0.25 µg of TNT was placed directly on another SDB-RPS extraction disk. The SPE disks were run on the IMS, and the peak intensities of TNT were recorded and compared. From the information obtained, the predicted concentration and percent recovery were then calculated.

Another common problem with SPE experiments is a phenomenon known as breakthrough. Breakthrough can occur when the analyte is not sufficiently retained on the sorbent. This would occur because the concentration of the analyte in the solution would be too high. All of the active sites on the SPE disk would be used, and the analyte would pass through the disk into waste because there would be no retention. This is called breakthrough.49 Breakthrough studies were conducted where the sample was first run through a SPE disk and then run through another SPE disk. The second SPE disk was then placed in the IMS. Because of the low sample concentrations, breakthrough did not occur until a concentration of analyte was reached that the spectrometer could detect without utilizing the SPE disks. Table 3.3 provides a comparison between the breakthrough concentrations for each explosive and its detection limit without extraction.
Table 3.4 Results of recovery analysis of explosives. Results are taken as the mean of three consecutive extractions from the same sample.

<table>
<thead>
<tr>
<th>Explosive</th>
<th>Spiked Solution (µg/L)</th>
<th>Predicted Concentration (µg/L)</th>
<th>% RSD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNT</td>
<td>0.50</td>
<td>0.49</td>
<td>6</td>
<td>99</td>
</tr>
<tr>
<td>RDX</td>
<td>0.50</td>
<td>0.49</td>
<td>10</td>
<td>97</td>
</tr>
<tr>
<td>HMX</td>
<td>0.50</td>
<td>0.47</td>
<td>24</td>
<td>93</td>
</tr>
</tbody>
</table>
These detection limits were determined by the detection of analyte peaks in the SIMPLISMA spectra.

River water samples were collected from the Hocking River, Athens, OH. Figure 3.22 gives SIMPLISMA spectra from a 500 mL sample of 1.0 µg/L TNT spiked river water. River water blanks not spiked with TNT produced the same peaks. Two peaks that might interfere with the detection of the explosives appeared at drift times of 14.2 and 16.6 ms with corresponding reduced mobilities of 1.49 and 1.27 cm²V⁻¹s⁻¹, respectively. The peak at a $K_0$ of 1.49 cm²V⁻¹s⁻¹ is very similar to the TNT peak with a $K_0$ of 1.45 cm²V⁻¹s⁻¹. Figure 3.23 gives the results after extraction and shows that utilizing SPE not only allows for lower detection limits but also removed the interfering peaks from the spectra. By comparing Figure 3.23, Figure 3.24, and Figure 3.25 the reproducibility of this method can be seen. All three of these experiments were conducted on the same day.

Coupling SPE with IMS produced a system with greater sensitivity and allowed for the detection of trace amounts of explosives in a liquid matrix, which under other circumstances would be difficult. It also produced a system with improved selectivity as a result of the removal of interferences with the addition of the extraction step. Utilizing SIMPLISMA allowed detection limits as low as a part per trillion for TNT with low part per trillion concentrations also achieved for RDX and HMX. This procedure would work well as a screening method because of the real-time, on-site monitoring capabilities and detection of explosives well under the EPA concentration guidelines.
Figure 3.22 SIMPLISMA extracted spectra of a 500 mL sample of TNT spiked river water at a concentration of 1.0 µg/L without solid phase extraction. This analysis was conducted isothermally at 275 ºC. The TNT peak is not present. A possible interference is present at a reduced mobility of 1.49 cm²V⁻¹s⁻¹. Components are ordered by SIMPLISMA purity value.
Figure 3.23 SIMPLISMA extracted spectra of a 500 mL sample of TNT spiked river water at a concentration of 1.0 µg/L after solid phase extraction. This analysis was conducted isothermally at 275 ºC. The interference peak has been removed, and the TNT peak is present. Components are ordered by SIMPLISMA purity value.
**Figure 3.24** SIMPLISMA extracted spectra of a 500 mL sample of TNT spiked river water at a concentration of 1.0 µg/L after solid phase extraction. This analysis was conducted isothermally at 275 °C. This figure should be compared to Figure 3.23 and Figure 3.25 to show reproducibility. Components are ordered by SIMPLISMA purity value.
Figure 3.25 SIMPLISMA extracted spectra of a 500 mL sample of TNT spiked river water at a concentration of 1.0 µg/L after solid phase extraction. This analysis was conducted isothermally at 275 ºC. This figure should be compared to Figure 3.23 and Figure 3.24 to show reproducibility. Components are ordered by SIMPLISMA purity value.
3.4. Detection of Illegal Narcotics and Their Metabolites in Urine

In the United States, drug testing is being carried out increasingly by employer screening programs for the workplace.\textsuperscript{73} Drug screening is important for employers and employees because an impaired workforce means unhealthy employees, unsafe working conditions, loss of productivity, smaller profits, more accidents, higher medical claims expenses, and a host of other negative effects for the employer and the employees.\textsuperscript{74} Federally regulated drug testing programs have protocols which involve an initial screen using immunoassay followed by a confirmatory GC-MS analysis on immunoassay-positive samples.\textsuperscript{75} Testing for drugs is usually performed using a urine sample.\textsuperscript{73}

The results to be presented in this chapter include the detection and identification of cocaine, cocaethylene, benzoylecgonine, ecgonine methyl ester, and (-)-$\Delta^9$-tetrahydrocannabinol (THC) in urine samples by solid phase extraction-ion mobility spectrometry (SPE-IMS)\textsuperscript{76}. Cocaine and marijuana are two of the most commonly abused drugs in the United States today with approximately 1.7 million individuals considered habitual cocaine abusers in 1998.\textsuperscript{77} However, marijuana remains the most commonly abused illicit drug in the United States according to data collected by the National Household Survey on Drug Abuse (NHSDA) in 1998. More than 72 million Americans twelve years of age and older, almost 1/3 of the entire American population, have tried marijuana at least once in their lifetime, and almost 18.7 million had used marijuana in the past year.\textsuperscript{78}

Urine is the most commonly used biological fluid employed to detect the presence of abused drugs in preliminary testing. The choice of urine is due to many advantages
compared to other biological materials, such as blood, saliva, hair, and sweat. It is generally available in a large quantity and can be collected with a non-invasive method. Also, it has a longer detection time window for most drugs and contains high concentrations of most metabolites. Urine can also be initially tested using a point-of-collection method such as the immunoassay, or it is stable enough that it can be sent to a laboratory for analysis.

Benzoylecgonine and ecgonine methyl ester are metabolites of cocaine, or products that appear as cocaine undergoes reactions in the body, and are generally excreted in the urine, sweat, and saliva. When cocaine is ingested with alcohol, another metabolite, cocaethylene, is produced in the liver causing intensified euphoric effects. The Substance Abuse and Mental Health Services Administration (SAMHSA) mandatory guidelines for federal workplace drug testing programs sets the initial and confirmation test cut-off level for cocaine metabolites in urine at 300 ng/mL and 150 ng/mL, respectively. Benzoylecgonine is typically the metabolite most commonly monitored. (-)-Δ⁹-THC, or the main component of marijuana, was also investigated in this study. The initial and confirmation test cut-off levels for metabolites of (-)-Δ⁹-THC in urine are set at 50 ng/mL and 15 ng/mL, respectively. The standard in workplace drug testing was established by the U.S. Department of Health and Human Services (DHHS) in 1988. This method includes an immunoassay screen followed by the GC-MS confirmation conducted on a urine sample. The immunoassay screen is conducted at the point-of-collection with the sample being sent to a laboratory for the confirmation GC-MS test. Despite widespread application, immunoassay kit responses are susceptible
to interferences caused by the presence of prescription drugs and adulterants in the urine. Results do not differentiate between metabolites and give only minimal quantification of the analyte.\textsuperscript{85} Therefore, a fast, on-site, non-expensive screening method that is not susceptible to interferences and adulterants is needed.

\subsection{3.5. Experimental Data for Narcotics and Metabolites}

The ion mobility spectrometers used in this work were the Barringer Ionscan\textsuperscript{®} Model 350 (Barringer Instruments Inc., New Jersey, USA) and the Ion Track Itemiser\textsuperscript{®} (Ion Track Instruments, Massachusetts, USA). The majority of the experiments for this work were conducted with the Barringer Ionscan\textsuperscript{®} Model 350. The Ion Track Itemiser\textsuperscript{®} was used to determine if results were reproducible on a different spectrometer. All spectra were acquired in positive ion, or narcotics, mode.

For the work conducted with the Ionscan\textsuperscript{®} Model 350, nicotinamide was the internal calibrant used in positive ion mode, and the structure for nicotinamide is given in Figure 3.26. Each spectrum comprised 1600 data points. The data acquisition frequency was 80 kHz. Data were collected on a National Instruments\textsuperscript{TM} AT-MIO-16XE-10 board located in a Pentium Pro 200 MHz computer, and the acquisition system was a homebuilt LabVIEW 6i virtual instrument. Spectra were collected after a 5.00-millisecond delay with a shutter grid width of 200 \(\mu\)s. The scan period was set at 20 milliseconds, and desorption time was set at 20 s. The drift flow was 300 cm\(^3\)/min with a 228 cm\(^3\)/min-sample flow, which resulted in an exhaust flow of 528 cm\(^3\)/min for the spectrometer.

The inlet temperature and drift tube temperature remained constant throughout the experiments at 294 and 233 °C, respectively. The desorber heater is usually held
Figure 3.26  Structure of positive ion mode calibrant, nicotinamide.
constant at 280 °C for the detection of narcotics. For this experiment, different
desorption temperatures (100°C - 300°C) were applied to determine the optimal
temperature needed to extract the narcotic from the SPE disks. All measurements used
isothermal desorption, and the optimal desorption temperature was found to be 250 °C.
This temperature was the highest possible temperature that did not destroy the SPE disk.

A schematic of the Ion Track Instruments Itemiser® ion mobility spectrometer is
given in Figure 3.27. Many of the principles applied for the Ionscan® Model 350 hold for
the Itemiser® as well; however, there are several differences. First, the Itemiser®
employs a sample trap in much the same way the Ionscan® Model 350 utilizes a sample
filter. The sample trap is dropped into the detection slot on the vapor desorption unit.
The sample trap is heated and desorbed vapors are drawn into the detection system. The
Itemiser® also utilizes a semi-permeable, elastomeric membrane that only allows target
vapors to permeate through into the detection system. The membrane prevents dust and
dirt from contaminating the detector. The sample molecules that pass through the
membrane are carried into the detector on a stream of clean, dry air. The gas flows
through an ionization chamber where the molecules are ionized to form both positive and
negative ions by a ⁶³Ni radioactive foil. The sample ions are then allowed to exit through
an open grid electrode into the ion drift region of the spectrometer by a small circulating
pump. The detector’s drift region emits short pulses across the electric field of the
chamber. This pulsed electric field forces the sample to proceed toward the collector
electrode. The speed of the ion is related to its size, thus the ions are separated based on
their size.⁸⁶
Figure 3.27  Schematic of ion mobility spectrometer adapted from reference 86.  The circles indicate the formation of vapors and ions that are separated based on size and charge in the drift tube.
For the work conducted with the Itemiser®, each spectrum was made up of 1600 data points. The data acquisition frequency was 80 kHz. Data are collected on a National Instruments™ DAQCard-Al-16XE-50 located in a Gateway Pentium III 400 MHz laptop computer, and the acquisition system was a homebuilt LabVIEW 6.1 virtual instrument. The scan period was set at 20 milliseconds, and desorption time was set at 5 s. The sample flow through the spectrometer is 1 L/min. Analysis time was set at 7 s.

Standard solutions of cocaine (Lot 35006-18A), cocaethylene (Lot 31555-09C), benzoylecgonine (Lot 32469-49G), ecgonine methyl ester (Lot 31543-77E), and (-)-Δ⁹-THC (Lot 34768-90A) in methanol were purchased from Radian Analytical Products. The cocaine, cocaethylene, and (-)-Δ⁹-THC concentrations were 1.0 mg/mL. The benzoylecgonine and ecgonine methyl ester concentrations were 100 µg/mL. To determine experimental reduced mobilities, a 1 µL sample of the narcotic was placed on an IMS filter disk. The disk was then heated by the desorber heater and analyzed by IMS. Structures of these compounds are given in Figure 3.28 and Figure 3.29. Experimental $K_0$ values were used to identify unknown peaks during the course of the experiments and are presented in Table 3.5. Precision of these reduced mobilities is based on the change in the reduced mobility of the nicotinamide peak. The nicotinamide peak reduced mobility for this project was $1.86±0.04\text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ with the error reported as the 95% confidence limit. This was determined by taking five samples over the course of the experiments. Known concentration solutions of all of the above narcotics and metabolites were also prepared in human urine provided by a twenty-six year old female for experimental data. These solutions were made by adding the standard narcotic.
Figure 3.28 Structures for (A) cocaine and its metabolites, (B) benzoylegonine, (C) ecgonine methyl ester, and (D) cocaethylene.
**Figure 3.29** Structures for (A) (-)-$\Delta^9$-tetrahydrocannobinal and its metabolite, (B) (-)-11-nor-9-carboxy-$\Delta^9$-tetrahydrocannabinol.
Table 3.5  Narcotics and their metabolites investigated with solid phase extraction ion mobility spectrometry with their molecular masses, experimental reduced mobilities, and reduced mobilities provided by (A)Reference 16 and (B) Iontrack Instruments.\textsuperscript{86}

Precision of these reduced mobilities is based on the change in the reduced mobility of the nicotinamide peak which was $1.86\pm0.04 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ with the error reported as the 95\% confidence limit. This was determined by taking five samples over the course of the entire project.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Molecular Mass (g/mol)</th>
<th>Experimental $K_0$ (cm$^2$V$^{-1}$s$^{-1}$)</th>
<th>Literature $K_0$ (cm$^2$V$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>303.4</td>
<td>1.16</td>
<td>1.16$^A$</td>
</tr>
<tr>
<td>Benzoylcegonine</td>
<td>289.4</td>
<td>1.19</td>
<td>1.19$^B$</td>
</tr>
<tr>
<td>Ecgonine Methyl Ester</td>
<td>199.4</td>
<td>1.56</td>
<td>1.56$^B$</td>
</tr>
<tr>
<td>Cocaethylene</td>
<td>317.4</td>
<td>1.13</td>
<td>1.13$^B$</td>
</tr>
<tr>
<td>(-)-$\Delta^9$-THC</td>
<td>314.5</td>
<td>1.05</td>
<td>1.05$^A$</td>
</tr>
</tbody>
</table>
solution to the 10 mL of urine. The concentrations presented in the figures of this chapter are 300 ng/mL for cocaine and its metabolites and 50 ng/mL for (-)-\(\Delta^9\)-THC.

Teflon IMS sample filters (Barringer Instruments), Part No. PL09045, were used to collect samples for insertion into the IMS for standard solution runs on the Ionscan® Model 350, and contraband sample traps (Ion Track Instruments), Part No. M0001140-C, were used to collect samples for standard runs on the Itemiser®. Several commercially available SPE disks were investigated in this work including 3M Empore™ SDB-RPS, C-8, and C-18 high performance extraction disks. C-8 disks were found to produce the best results and were therefore chosen as the disks to use to extract samples for insertion into the IMS for the SPE runs. Table 3.6 compares results obtained with different disks for cocaine and (-)-\(\Delta^9\)-THC. Conditioning of the SPE disks were conducted according to instructions provided with the disks. The procedure utilized to clean and condition the C-8 extraction disk is as follows. A SPE disk was placed in a reservoir and a vacuum was applied to pull the cleaning and conditioning solvents through the disk. First, the disk was cleaned by adding 10 mL of methanol to the reservoir above the disk. Then, 10 mL of reagent grade water was added to the reservoir and pulled through the disk. After the disk dried, the disk was conditioned by adding 5 mL of methanol to the reservoir. When the methanol level reached approximately 1 mm above the disk, 10 mL of reagent grade water was added to the reservoir. When the water level reached approximately 1 mm above the disk, 10 mL of prefiltered narcotic spiked urine sample was run through the SPE disk. The urine was prefiltered to prevent clogging of the SPE disk. VWR® filter
Table 3.6 Comparison of narcotic peak height results with extraction by 3M Empore™ SDB-RPS, C-8, and C-18 solid phase extraction disks on the Barringer Ionscan® Model 350. Peak height in units of volts is reported as an average of three runs with the 95% confidence limit.

<table>
<thead>
<tr>
<th>Narcotic</th>
<th>High Performance SPE Disk</th>
<th>Peak Height (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>SDB-RPS</td>
<td>NA</td>
</tr>
<tr>
<td>(50 ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-8</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>C-18</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>(-)-Δ⁹-THC</td>
<td>SDB-RPS</td>
<td>NA</td>
</tr>
<tr>
<td>(50 ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-8</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>C-18</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

NA indicates the absence of a peak.
paper, Cat. No. 28321-099, was used to filter urine specimens. Narcotic spiked urine samples were also poured through IMS fiberglass sample filters for comparison. The disk was dried before IMS analysis was conducted. HPLC grade methanol was purchased from Fischer Scientific, Lot No. 001927.

Experiments were conducted with several possible drug and metabolite combinations and results obtained from both instruments were compared. Experiments were also conducted with the addition of common adulterants including ammonia, bleach, Drano, and detergent. The result provided for adulterant analysis is the addition of 0.5 mL of bleach to the narcotic spiked urine sample.

3.6. Results of Narcotics and Metabolites Experiments

Figure 3.30 gives a SIMPLISMA extracted positive ion spectra of 1 µL of cocaine at a concentration of 1.0 mg/mL in methanol. In this figure, the nicotinamide and cocaine peaks appear at drift times of approximately 9.4 and 15.1 ms, respectively. These drift times correspond to reduced mobilities of 1.86 and 1.16 cm²V⁻¹s⁻¹. The ordinate corresponds to the relative intensities of the peaks, while the abscissa corresponds to the drift time measurement. As cocaine is metabolized by the human body, oxidation products are formed that are soluble in urine. Two of the main metabolites of cocaine are benzoylecgonine and eegonine methyl ester.

The SIMPLISMA extracted spectra for 1 µL of benzoylecgonine and eegonine methyl ester at concentrations of 100 µg/mL in methanol are given in Figure 3.31 and Figure 3.32, respectively. The benzoylecgonine peak, according to Figure 3.31, appears at approximately 14.7 ms with a corresponding \( K_0 \) of 1.19 cm²V⁻¹s⁻¹. Benzoylecgonine
Figure 3.30 SIMPLISMA extracted spectra of a 1 µL sample of cocaine at a concentration of 1.0 mg/mL in methanol. This analysis was conducted isothermally at 280 °C. Components are ordered by SIMPLISMA purity value.
Figure 3.31 SIMPLISMA extracted spectra of a 1 µL sample of benzoylecgonine at a concentration of 100 µg/mL in methanol. This analysis was conducted isothermally at 280 °C. Benzoylecgonine is the main metabolite of cocaine found in urine. Components are ordered by SIMPLISMA purity value.
Figure 3.32 SIMPLISMA extracted spectra of a 1 µL sample of ekgonine methyl ester at a concentration of 100 µg/mL in methanol. This analysis was conducted isothermally at 280 °C. Ecgonine methyl ester is a metabolite of cocaine found in urine. Components are ordered by SIMPLISMA purity value.
differs from cocaine in the loss of a \(-\text{CH}_2\) group, and this explains why the peak appears at a lower drift time and larger $K_0$ than cocaine. Figure 3.32 gives the SIMPLISMA extracted spectra of a 1$\mu$L sample of ecgonine methyl ester at a concentration of 100 $\mu$g/mL in methanol. The ecgonine methyl ester peak appears at a drift time of approximately 11.3 ms with a corresponding $K_0$ of 1.56 cm$^2$V$^{-1}$s$^{-1}$. Ecgonine methyl ester differs from cocaine in the loss of a \(-\text{COC}_6\text{H}_5\) group, which is a substantial decrease in size and results in a significantly decreased drift time and a much larger $K_0$.

Cocaethylene, a byproduct produced when cocaine and alcohol undergo reactions in the body, results when individuals ingest alcohol concurrently with cocaine. Cocaethylene differs from cocaine in the addition of a \(-\text{CH}_2\) group. Figure 3.33 gives the SIMPLISMA extracted spectra for a 1 $\mu$L sample of cocaethylene at a concentration of 1.0 mg/mL in methanol. The cocaethylene peak appears at a drift time of 15.5 ms. The resulting $K_0$ is 1.13 cm$^2$V$^{-1}$s$^{-1}$. Cocaethylene differs from cocaine in the addition of a \(-\text{CH}_2\) group. This addition explains why the cocaethylene peak appears at a longer drift time and smaller $K_0$ than cocaine.

Another highly abused drug in the U.S. is marijuana. The active ingredient in marijuana is (-)-$\Delta^9$-THC. Figure 3.34 gives a SIMPLISMA extracted spectra of a 1 $\mu$L sample of (-)-$\Delta^9$-THC at a concentration of 1.0 mg/mL in methanol. The (-)-$\Delta^9$-THC appears at a drift time of approximately 16.7 ms with a resulting $K_0$ of 1.05 cm$^2$V$^{-1}$s$^{-1}$. The main metabolite of (-)-$\Delta^9$-THC is (-)-11-nor-9-carboxy-$\Delta^9$-THC. The structure for this compound is given in Figure 3.29. (-)-11-nor-9-carboxy-$\Delta^9$-THC is the compound investigated in drug screening of urine. Unfortunately, the (-)-11-nor-9-carboxy-$\Delta^9$-THC
Figure 3.33 SIMPLISMA extracted spectra of a 1 μL sample of cocaethylene at a concentration of 1.0 mg/mL in methanol. This analysis was conducted isothermally at 280 °C. Cocaethylene is a byproduct produced by the body when cocaine and alcohol are ingested together. Components re ordered by SIMPLISMA purity value.
Figure 3.34 SIMPLISMA extracted spectra of a 1 µL sample of (-)-Δ⁹-THC at a concentration of 1.0 mg/mL in methanol. This analysis was conducted isothermally at 280 °C. Components are ordered by SIMPLISMA purity value.
did not produce an IMS response, because it is not as basic as the nicotinamide or ammonium reagent ions and cannot ionize with these reagent ions. It is believed that derivatization of the compound is necessary to produce peaks in IMS.\textsuperscript{88} (-)-\textDelta^9-THC is present in blood and plasma after ingestion at concentrations of greater than 1 ng/mL and 2-3 ng/mL, respectively.\textsuperscript{78} Studies were conducted with (-)-\textDelta^9-THC in urine to determine if the extraction of (-)-\textDelta^9-THC from a biological fluid was possible by this method.

For the SPE experiments, dilutions of cocaine, benzylecgonine, ecgonine methyl ester, cocaethylene, and (-)-\textDelta^9-THC were made in urine by addition of the purchased standard solutions. The C-8 extraction disks were cleaned and conditioned before sample extraction. The disks were cleaned by wetting with several milliliters of methanol and allowing the disks to dry. Then, the disks were wetted with 10 mL of reagent grade water and allowed to dry. Finally, the disks were conditioned with 5 mL of methanol followed by 10 mL of reagent grade water. The urine was filtered through filter paper before the extraction procedure. This was done to remove any large particles in the urine. Ten milliliters of the filtered, spiked urine sample was passed through the solid phase extraction disk. Samples were also poured through IMS Teflon sample filters for comparison. The disks were dried before placing them above the IMS desorber heater. Peaks appeared for all narcotic and metabolite compounds at lower concentrations with the SPE disks than with the IMS filters.

For this work, figures will be shown at the initial screening level. Detection at lower concentrations was possible as evidenced by the detection limits provided in Table 3.7. Detection limits were calculated by taking three times the standard deviation of the
Table 3.7 Comparison of detection limits without solid phase extraction and detection limits after solid phase extraction of narcotics and metabolites on the Barringer Ionscan® Model 350.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Detection Limit without SPE (µg/mL)</th>
<th>Detection Limit after SPE (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>1.0</td>
<td>0.30</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>7.0</td>
<td>0.95</td>
</tr>
<tr>
<td>Ecgonine Methyl Ester</td>
<td>12</td>
<td>1.6</td>
</tr>
<tr>
<td>Cocaethylene</td>
<td>10</td>
<td>1.1</td>
</tr>
<tr>
<td>(-)-∆⁹-THC</td>
<td>6.0</td>
<td>0.90</td>
</tr>
</tbody>
</table>
noise. Samples at the detection limit concentration were run to verify the presence of an analyte peak. However, IMS would be utilized as the initial screening method for illegal narcotics and their metabolites in urine. Confirmation analysis would still be necessary.

One problem with current initial screening methods is the inability to detect two compounds simultaneously. Figure 3.35 gives the results of the experimental run of a 10 mL mixture of benzoylecgonine and ecgonine methyl ester spiked urine at a concentration of 300 ng/mL by using IMS filter disk and no extraction. From this figure, there are peaks for the nicotinamide and urine. The compound responsible for the urine peak completely suppressed the benzoylecgonine and ecgonine methyl ester. This explains why these two compounds were not found. Figure 3.36 gives the results of the experimental run of a 10 mL mixture of benzoylecgonine and ecgonine methyl ester spiked urine at a concentration of 300 ng/mL with SPE. Benzoylecgonine and ecgonine methyl ester are the two most commonly screened metabolites when testing for cocaine usage. By utilizing SPE, the sample was benzoylecgonine and ecgonine methyl ester were retained on the disk while the urine compound was not. Therefore, the detection of benzoylecgonine and ecgonine methyl ester was possible. Figure 3.37 and Figure 3.38 show two other 10 mL mixtures of benzoylecgonine and ecgonine methyl ester spiked urine at concentrations of 300 ng/mL with SPE. Comparing these three figures shows the reproducibility of the measurement.

Figure 3.39 provides the SIMPLISMA concentration profile for this experiment. At the beginning of the experiment, the concentration of the nicotinamide is high while the concentrations of the two metabolites are at zero. When the sample is first introduced
Figure 3.35 SIMPLISMA extracted spectra on the Barringer Ionscan® Model 350 of a 10 mL mixture of benzoylecgonine and ecgonine methyl ester at concentrations of 300 ng/mL in urine by utilizing IMS filter disks. This analysis was conducted isothermally at 250 °C. The urine completely suppressed the benzoylecgonine and ecgonine methyl ester. A peak is present for urine, but no peaks are present for benzoylecgonine or ecgonine methyl ester. Components are ordered by SIMPLISMA purity value.
Figure 3.36 SIMPLISMA extracted spectra on the Barringer Ionscan® Model 350 of a 10 mL mixture of benzoylecgonine and ecgonine methyl ester at concentrations of 300 ng/mL in urine after solid phase extraction. This analysis was conducted isothermally at 250 °C. The extraction procedure removed the urine interferent, and the two analyte peaks are present in the spectra. Components are ordered by SIMPLISMA purity value.
Figure 3.37 SIMPLISMA extracted spectra on the Barringer Ionscan® Model 350 of a 10 mL mixture of benzoylcegonine and ecgonine methyl ester at concentrations of 300 ng/mL in urine after solid phase extraction. This analysis was conducted isothermally at 250 °C. This figure should be compared to Figure 3.36 and Figure 3.38 to show reproducibility. Components are ordered by SIMPLISMA purity value.
**Figure 3.38** SIMPLISMA extracted spectra on the Barringer Ionscan® Model 350 of a 10 mL mixture of benzoylecgonine and ecgonine methyl ester at concentrations of 300 ng/mL in urine after solid phase extraction. This analysis was conducted isothermally at 250 °C. This figure should be compared to Figure 3.36 and Figure 3.37 to show reproducibility. Components are ordered by SIMPLISMA purity value.
Figure 3.39  SIMPLISMA concentration profiles on the Barringer Ionscan® Model 350 of a 10 mL mixture of benzoylecgonine and ecgonine methyl ester at concentrations of 300 ng/mL in urine after solid phase extraction. This analysis was conducted isothermally at 250 °C. The rate of growth and decay for the benzoylecgonine and ecgonine methyl ester peaks are different. Components are ordered by SIMPLISMA purity value.
into the spectrometer, the nicotinamide peak decreased rapidly as charge was transferred to the analytes. The ecgonine methyl ester peak increased and then decreased rapidly while the benzoylcegonine peak gradually increased toward the end of the experiment. At the end of the twenty second experiment, the analyte concentrations return to zero as the charge is transferred back to the nicotinamide. The nicotinamide peak gradually returns to its original intensity.

Figure 3.40 gives the SIMPLISMA extracted positive ion spectra for a 10 mL sample of cocaethylene at a concentration of 300 ng/mL in urine after SPE on the Iontrack Itemiser®. By comparing Figure 3.40 with Figure 3.41 and Figure 3.42, the reproducibility of this measurement can be seen. The Itemiser® produced similar results in all experiments to the Ionscan® Model 350. Two advantages were evident when utilizing the Itemiser®. First, the Itemiser® employed ammonia as a dopant for positive ion mode to work much the same way the hexachloroethane works in negative ion mode on the Ionscan® Model 350. However, the ammonia did not appear to improve the detection of the compounds investigated in this work. Second, the Itemiser® allows the user to visualize the entire concentration profile as evidenced in the SIMPLISMA concentration profile given in Figure 3.43. The instrument does not stop collecting data at the end of the sample analysis time. In the Ionscan® Model 350, the instrument stops collection of data at the end of the twenty second experiment by closing the gating grid. This allows the instrument to clear down at a faster rate. However, important temporal information could possibly be lost. The Itemiser® also has several apparent disadvantages when compared to the Ionscan® Model 350. The Itemiser® does not
Figure 3.40 SIMPLISMA extracted spectra on the Ion Track Itemiser® of a 10 mL sample of cocaethylene at a concentration of 300 ng/mL in urine after solid phase extraction. This analysis was conducted isothermally at 250 °C. The extraction procedure removed the urine interferent, and the cocaethylene peak is present in the spectra. The reactant ion peak is the background signal. Components are ordered by SIMPLISMA purity value.
Figure 3.41 SIMPLISMA extracted spectra on the Ion Track Itemiser® of a 10 mL sample of cocaethylene at a concentration of 300 ng/mL in urine after solid phase extraction. This analysis was conducted isothermally at 250 °C. This figure should be compared to Figure 3.40 and Figure 3.42 to show reproducibility. Components are ordered by SIMPLISMA purity value.
Figure 3.42 SIMPLISMA extracted spectra on the Ion Track Itemiser® of a 10 mL sample of cocaethylene at a concentration of 300 ng/mL in urine after solid phase extraction. This analysis was conducted isothermally at 250 °C. This figure should be compared to Figure 3.40 and Figure 3.41 to show reproducibility. Components are ordered by SIMPLISMA purity value.
Figure 3.43 SIMPLISMA concentration profile on the Ion Track Itemiser® of a 10 mL sample of cocaethylene at a concentration of 300 ng/mL in urine after solid phase extraction. This analysis was conducted isothermally at 250 °C. The rate of growth and decay for the cocaethylene peak is shown. Components are ordered by SIMPLISMA purity value.
employ an internal calibrant, so standard samples had to be run in between experiment of unknown samples.

The optimal desorption temperature necessary to extract the samples from the disk was determined to be 250 °C. At higher temperature, thermal decomposition of the C-8 extraction disks resulted. When the temperature of desorption was too high, the white extraction disks would turn brown and black indicating the destruction of the disk. However, if the temperature was not sufficiently high, the analytes would not completely desorb from the SPE disk. An optimal temperature of 250 °C was determined. This temperature was the highest possible temperature that did not destroy the C-8 extraction disk. Multiple runs on the IMS were conducted at this temperature with the same disk to ensure repeatability.

Recovery experiments were investigated at this 250 °C, and the results are given in Table 3.8. Because of the problems associated with quantitation in IMS, recovery experiments were conducted by comparing peaks after solid phase extraction for a dilute solution with peaks obtained by placing an equivalent amount of explosive directly on the solid phase disk. The concentration of samples used in the recovery experiments was much lower than the initial screening concentrations. The reason for utilizing lower concentrations for recovery analysis was because at the initial screening levels, the samples are at a high concentration. For a more accurate interpretation of the results, lower concentrations were utilized. An example of how the recovery experiments were conducted follows. A 10 mL sample of cocaine spiked urine at a concentration of 50 ng/mL was run through a C-8 extraction disk, and 500 ng of cocaine should be retained
Table 3.8  Results of recovery analysis of narcotics and their metabolites on the Barringer Ionscan® Model 350. Results are taken as the mean of three consecutive extractions from the same sample.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Spiked Solution (ng/mL)</th>
<th>Predicted Concentration (ng/mL)</th>
<th>% RSD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>50</td>
<td>49</td>
<td>5</td>
<td>98</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>50</td>
<td>47</td>
<td>13</td>
<td>95</td>
</tr>
<tr>
<td>Ecgonine Methyl Ester</td>
<td>50</td>
<td>46</td>
<td>14</td>
<td>93</td>
</tr>
<tr>
<td>Cocaethylene</td>
<td>50</td>
<td>49</td>
<td>10</td>
<td>98</td>
</tr>
<tr>
<td>(-)-Δ⁹-THC</td>
<td>50</td>
<td>46</td>
<td>10</td>
<td>93</td>
</tr>
</tbody>
</table>
on the extraction disk. Therefore, 500 ng of cocaine was placed directly on another C-8 extraction disk. The SPE disks were run on the IMS, and the peak intensities of cocaine were recorded and compared. From the information obtained, the predicted concentration and percent recovery were then calculated.

A possible problem for the detection of illegal narcotics in urine is when adulterants are added to the urine specimens in the hope of masking the presence of narcotics and metabolites. Several adulterants were investigated including ammonia, bleach, Drano, and detergent. Figure 3.44 gives the SIMPLISMA extracted spectra of a 10 mL sample of (-)-Δ⁹-THC at a concentration of 50 ng/mL in urine with the addition of 0.5 mL of bleach without SPE. The peaks resulting from bleach can be seen at between 7.5 to 10 ms. The nicotinamide peak is present at 9.4 ms along with a peak resulting for the urine at 10.2 ms. The resulting reduced mobilities are 1.86 and 1.72 cm²V⁻¹s⁻¹, respectively. There is no evident (-)-Δ⁹-THC peak. However, following SPE, the (-)-Δ⁹-THC peak appear at a drift time of 16.7 ms and a $K_0$ of 1.05 cm²V⁻¹s⁻¹. See Figure 3.45. Also, the peaks resulting from urine and bleach are no longer visible. Figure 3.46 and Figure 3.47 give the results from two other experiments conducted on the same day.

Coupling SPE with IMS produced a system that allowed for the detection of proscribed narcotics and several metabolites in urine below the screening cut-off levels determined by the SAMHSA. Improved detections were also achieved with the coupling of the extraction step. The addition of adulterants to the urine did not affect the resulting IMS spectra after SPE was applied to remove the analytes from the urine.
Figure 3.44  SIMPLISMA extracted spectra on the Barringer Ionscan® Model 350 of a 10 mL sample of (-)-$\Delta^9$-THC at a concentration of 50 ng/mL in urine with the addition of 5% bleach and no solid phase extraction. This analysis was conducted isothermally at 250 °C. The urine and the bleach act as interferents and suppress the (-)-$\Delta^9$-THC. Components are ordered by SIMPLISMA purity value.
Figure 3.45 SIMPLISMA extracted spectra on the Barringer Ionscan® Model 350 of a 10 mL sample of (-)-Δ⁹-THC at a concentration of 50 ng/mL in urine with the addition of 5% bleach after solid phase extraction. This analysis was conducted isothermally at 250 °C. The urine and bleach have been removed due to the extraction and the (-)-Δ⁹-THC is present. Components are ordered by SIMPLISMA purity value.
Figure 3.46 SIMPLISMA extracted spectra on the Barringer Ionscan® Model 350 of a 10 mL sample of (-)-Δ⁹-THC at a concentration of 50 ng/mL in urine with the addition of 5% bleach after solid phase extraction. This figure should be compared to Figure 3.45 and Figure 3.47 to show reproducibility. Components are ordered by SIMPLISMA purity value.
**Figure 3.47** SIMPLISMA extracted spectra on the Barringer Ionscan® Model 350 of a 10 mL sample of (-)-Δ⁹-THC at a concentration of 50 ng/mL in urine with the addition of 5% bleach after solid phase extraction. This figure should be compared to Figure 3.45 and Figure 3.46 to show reproducibility. Components are ordered by SIMPLISMA purity value.
3.7. **Discussion and Conclusion for SPE-IMS**

Coupling SPE with IMS enhanced the advantages of both techniques. The SPE concentrated and purified samples allowing large volume aqueous samples at trace concentrations to be investigated by IMS. IMS is a portable technique, so on-site analysis is also possible.
Chapter 4. Thermal Hydrolysis / Methylation Ion Mobility Spectrometry

The rapid and sensitive detection of microbes is necessary for an instrument to be valuable for detecting germ warfare agents. Bacterial cells produce lipids, and the correlation between bacterial lipid composition and taxonomic classification has been extensively used for the identification of bacteria. A new technique for the rapid screening of bacteria was developed in this work. Bacterial cells are not volatile, and by themselves cannot be easily identified by IMS. However, the method used in this work allows the identification of bacteria by one of their byproducts, lipids. Therefore, a new method was developed for the detection and identification of bacteria using IMS.

Standard samples of each bacterium were run separately to determine characteristic peaks that could be used for identifying a bacterium. Such characteristic peaks are referred to as biomarkers. Bacterial whole cells of each of the bacteria under investigation were placed on different IMS sample filters along with 1 µL of 0.1 M tetramethylammonium hydroxide (TMAH). The sample is thermally hydrolyzed by the desorber heater of the ion mobility spectrometer. The TMAH then methylates the lipids and produces substances known as fatty acid methyl esters, or FAMEs, which are volatile and can be identified by IMS. This whole process is known as thermal hydrolysis/methylation, or THM. Figure 4.1 gives a scheme of the THM process. The FAMEs are introduced into the spectrometer and produce ions that furnish peaks at characteristic mobilities. The bacteria were then classified by their FAME profiles using SIMPLISMA and PCA (Principal Component Analysis).
Figure 4.1  Scheme utilized to produce fatty acid methyl esters from bacteria whole cells. Cells are placed on an IMS filter disk with 1 µL of TMAH. The IMS filter disk is heated by the desorber heater, and the lipids produced by the bacteria are methylated to form fatty acid methyl esters.
4.1. **Food Poisoning Bacteria**

*Listeria monocytogenes* and *Bacillus cereus* are pathogenic (disease-causing) bacteria that can be found on food. *Listeria innocua* and *Listeria seeligeri* are the non-pathogenic forms of Listeria and are harmless to humans. This project aimed to identify the pathogenic form of Listeria in the presence of non-pathogenic Listeria and identify the different species present. It also intended to differentiate between different types of pathogenic bacteria.

*Listeria monocytogenes* has become an important food-borne pathogen since the 1980s when it was realized that it could be spread in food. Listeria is most commonly found in ready-to-eat products such as hot dogs, deli meats, and cheeses. The Food Safety and Inspection Service (FSIS) supported over 30 recalls of food products in the year 2000 and 25 recalls in 1999 because of *Listeria monocytogenes* contamination. These recalls included such products as ham, turkey and chicken products, sausage, beef jerky, and ice cream. Unfortunately, this bacterium can survive and grow in adverse conditions. The key problem is that *Listeria monocytogenes* can continue to grow and reproduce in refrigeration conditions that arrest the growth of other pathogens. *Listeria monocytogenes* can cause a potentially deadly disease called listeriosis, which can take as long as one to six weeks after ingestion to develop. This disease primarily affects pregnant women, newborns, people with weakened immune systems, and those over 60 years of age. Listeriosis is a flu-like illness with symptoms including fever, chills, fatigue, nausea, and diarrhea. If not identified quickly, listeriosis can lead to bacterial meningitis and death. Approximately 500 people die annually in the United States from
this infection\textsuperscript{90}, which is a mortality rate of approximately 20\% of those infected with the disease.\textsuperscript{91} The other species of Listeria investigated do not have any known adverse effects on humans.

A rapid screening technique is necessary for several reasons. Two standard methods for the detection of Listeria, accepted by the FSIS, include sampling, incubation, and pulsed-field gel electrophoresis (PFGE) and sampling, polymerase chain reaction, and PFGE.\textsuperscript{90} The first process is very lengthy, 24 to 48 hours, while the second process is not as time-consuming taking only several hours to produce results. The time and work necessary to use either of these techniques is detrimental because smaller quantities of food are actually tested, and the food cannot be distributed until the test results are reported. Another problem with these techniques is that non-pathogenic forms of Listeria cannot always be identified and separate tests must be conducted to identify different pathogenic bacteria. The ultimate goal of this work is to develop a screening technique that is beneficial in that more samples can be monitored in less time and at a lower cost than current methods for the detection of \textit{Listeria monocytogenes}. In addition, this method could be amenable to onsite inspection of the food processing plants.

\textit{Bacillus cereus} was also investigated in this work, and it has been linked to food poisoning outbreaks since the early 1950s. Meats, fish, milk, rice, pasta, cheese, puddings, sauces, soups and salads have all been implicated in \textit{B. cereus} outbreaks. The number of reported incidents of \textit{Bacillus cereus} foodborne illness ranges from 6 to 50 incidents per year. However, the occurrence of illnesses may be much greater. People may become ill from this type of foodborne illness and wait for symptoms to pass. They
usually do not consult a doctor and no stool specimens are analyzed for the presence of *B. cereus*. Bacillus cereus is normally present in many foods, but it must grow in the food to high numbers (greater than one million *Bacillus cereus* spores per gram of food) before it can cause illness. Therefore, controlling this organism is best done by preventing it from growing in foods. Hot foods should be held at temperatures above 130 degrees Fahrenheit, and cold foods should be held at temperatures below 40 degrees Fahrenheit. When hot foods must be cooled, they should be cooled rapidly.

*Bacillus cereus* causes two types of foodborne illness, and all types of people are susceptible. A diarrheal type of illness is caused by strains of *Bacillus cereus* that produce a high molecular weight diarrhea-genic toxin. A vomiting (emetic) type of illness is caused by strains that produce a low molecular weight emetic toxin. The diarrheal illness occurs six to fifteen hours after eating a food that is contaminated with high numbers of a toxigenic strain. The symptoms may include watery diarrhea, abdominal cramps and nausea, although vomiting is rare. The symptoms usually last for up to 24 hours. The vomiting illness occurs within 30 minutes to 6 hours after eating a food that is contaminated with high numbers of a toxigenic strain. The symptoms are nausea and vomiting, but may also include abdominal cramps and diarrhea. The symptoms usually subside in less than 24 hours. Generally, neither form of *Bacillus cereus* illness has been considered life-threatening.

A variety of methods have been recommended for the recovery, enumeration and confirmation of *Bacillus cereus* in foods. Unfortunately, at the current time there is no standard method for the detection of *Bacillus cereus* on foodstuffs prior to an outbreak.
Therefore, the detection and identification of *Bacillus cereus* on foodstuffs usually occurs after an outbreak of the disease. This work will also help detect, identify, and classify *Bacillus cereus* by a rapid, low-cost method.

4.2. **Experimental Data for Bacteria**

The ion mobility spectrometer used in this work was the Barringer Ionscan® Model 350 (Barringer Instruments Inc., New Jersey, USA). All spectra were acquired in positive ion, or narcotics, mode. For the work conducted with the Ionscan® Model 350, each spectrum comprised 1600 data points. The data acquisition rate was 80 kHz. Data are collected on a National Instruments™ AT-MIO-16XE-10 board located in a Pentium Pro 200 MHz computer, and the acquisition system was a homebuilt LabVIEW 6i virtual instrument. Spectra were collected after a 5.00-millisecond delay with a shutter grid width of 200 µs. The scan period was set at 20 milliseconds, and desorption time was set at 20 s. The drift flow was 300 cm³/min with a 228 cm³/min-sample flow. This resulted in an exhaust flow of 528 cm³/min for the spectrometer.

The inlet temperature and drift tube temperature remained constant throughout the experiments at 294 and 233 °C, respectively. The desorber heater is usually held constant at 280 °C for the detection of narcotics. For this experiment, a desorption temperature of 300°C was necessary to thermally hydrolyze the bacterial whole cells. All runs conducted were isothermal.

Freeze-dried *Listeria innocua* (#51742), *Listeria seeligeri* (#35967), *Listeria monocytogenes* (#19115), and *Bacillus cereus* (#11778) were purchased from ATCC. Bacteria were rehydrated by placing the freeze-dried pellet in 5 mL of brain-heart
infusion that had been autoclaved at 121 ºC for 15 minutes. Autoclaving ensures the sterility of the infusion and prevents growth of unwanted bacteria. The brain-heart infusion was purchased from Difco, Lot 9316001. The bacteria were allowed to rehydrate and grow in the infusion for 24 hours. After the 24 hours, several drops of the suspension of bacteria were inoculated on a brain-heart infusion agar plate. The agar was also autoclaved at 121 ºC for 15 minutes to ensure sterility. It was purchased from Sigma, Lot 90K0804. The bacteria were allowed to grow on the agar plate for 48 hours before experiments were conducted. Each sample for analysis was taken from a different plate of bacteria to prevent contamination and to show reproducibility. Therefore, many plates of bacteria were used over the course of these experiments. Plates of bacteria were visually compared with each other and ATCC descriptions. This was done to make sure that the bacteria were actually the bacteria under investigation.

Small amounts of bacteria were placed on Teflon IMS sample filters (Barringer Instruments), Part No. PL09045, for analysis by IMS. Tetramethylammonium hydroxide (TMAH) was utilized as the methylating agent, and 1 µL of TMAH was applied to the bacteria on the IMS sample filters. TMAH was purchased from Sigma, Lot 18H0443. Reduced mobilities for the THM bacteria investigated are given in Table 4.1. Precision of these reduced mobilities is based on the change in the reduced mobility of the nicotinamide peak. The nicotinamide peak reduced mobility for this project was 1.86 ±0.02 cm²V⁻¹s⁻¹ with the error reported as the 95% confidence limit. This was determined by taking five samples over the course of the entire project.
Table 4.1 Reduced mobilities obtained for bacteria experiments conducted with the addition of TMAH on the IMS filter disk. Precision of these reduced mobilities is based on the change in the reduced mobility of the nicotinamide peak which was 1.86±0.02 cm²V⁻¹s⁻¹ with the error reported as the 95% confidence limit. This was determined by taking five samples over the course of the entire project.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Reduced Mobility ($K_0$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMAH</td>
<td>2.38</td>
</tr>
<tr>
<td>Bacterial Components</td>
<td>1.71, 1.63</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>1.40, 1.35</td>
</tr>
<tr>
<td><em>Listeria innocua</em></td>
<td>1.98, 1.66, 1.53, 1.21</td>
</tr>
<tr>
<td><em>Listeria seeligeri</em></td>
<td>1.98</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>1.54, 1.47, 1.41</td>
</tr>
</tbody>
</table>
4.3. Results of Bacteria Experiments

Experiments were conducted with FAME standard, methyl laureate, to determine which mode needed to be utilized for this work. No results were obtained when negative ion experiments were conducted. Complex spectra resulted when positive ion mode experiments were conducted; therefore, all bacteria were investigated in positive ion mode.

Figure 4.2 gives a positive ion mode raw spectrum of a sample of *Bacillus cereus*. This analysis was conducted isothermally at 300 °C. One peak is apparent at a $K_0$ of 1.66 cm$^2$V$^{-1}$s$^{-1}$. This peak was present for all bacteria specimens sampled when no TMAH was applied to the IMS filter disk. It is possible that this peak is a result of the media instead of the bacteria since all bacteria were grown on the same media to eliminate the possibility of modeling differences in media instead of differences in bacteria.

Principal component analysis is a data analysis tool that can be utilized to detect relationships between variables, or classify variables. Principal component analysis involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called principal components. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. Figure 4.3 provides a graphical interpretation of PCA. The green circle encompasses the entire data. The red line is a vector that is drawn through the largest variance in the data set. Therefore, this vector is said to span the greatest variance in the data and is termed the first principal component. The blue line is a vector
Figure 4.2 Negative ion mode raw spectrum of *Bacillus cereus* on the Barringer Ionscan® Model 350 without addition of the methylating agent to the IMS filter. This analysis was conducted isothermally at 300 °C.
Figure 4.3 Description of principal component analysis. The green circle encompasses the entire data. Red vector indicates the first principal component, and the blue vector indicates the second principal component. The two vectors are orthogonal to one another and both vectors minimize the distances between the data objects and the projections of the objects onto each component.
that is drawn through the largest variance remaining in the data set at a right angle to the first principal component and is termed the second principal component. As many components can be utilized as necessary. For this work, the first two principal components were used. Figure 4.4 gives the 3D surface plot of the *Bacillus cereus* sample with the addition of 1 \( \mu \text{L} \) of TMAH. This analysis was conducted isothermally at 300 °C. This information, the raw spectra, was the information used for PCA analysis. Before utilizing PCA, the raw spectra containing only the spectra resulting from the nicotinamide or the TMAH were removed. This prevented classifying the results according to the nicotinamide or the TMAH instead of the FAME profiles.

Figure 4.5 gives the PCA results from bacterial samples with no methylation step from a sample of one experiment of each of *Bacillus cereus*, *Listeria innocua*, and *Listeria seeligeri*. An experiment consisted of taking bacteria from the plate, placing it on an IMS filter disk, and analyzing the disk by IMS. The analysis was conducted isothermally at 300 °C. This figure provides ion mobility spectral scores showing 40.4% of the cumulative variance between the first two principal components of the raw spectra from the bacteria samples investigated without the addition of the methylating agent. From this figure, there is no evidence of any classification.

When TMAH was added to the IMS filter disk along with the bacterial specimens and the sample was allowed to air dry, differences became apparent between the different bacteria. Figure 4.6 gives PCA results obtained when 1 \( \mu \text{L} \) of TMAH is added to the IMS filter disks along with each bacterial specimen. Each bacterial specimen was placed on a separate IMS filter disk. The analyses were conducted isothermally at 300 °C. This
Figure 4.4 Surface plot of a sample of *Bacillus cereus* on the Barringer Ionscan® Model 350 with the addition of 1µ L of methylating agent to the IMS filter. This analysis was conducted isothermally at 300 °C. The spectra that compose this plot were utilized to conduct PCA analysis on raw spectra.
Figure 4.5  Ion mobility spectral scores showing 40.4% of the cumulative variance between the first two principal components of the bacteria without the methylating step.

(B) Bacillus cereus, 201 raw spectra; (I) Listeria innocua, 202 raw spectra; (S) Listeria seeligeri, 150 raw spectra. From this figure, there is no evidence of classification.
Figure 4.6 Ion mobility spectral scores showing 40.2% of the cumulative variance between the first two principal components of bacteria after THM. (B) *Bacillus cereus*, 101 raw spectra; (I) *Listeria innocua*, 300 raw spectra; (S) *Listeria seeligeri*, 230 raw spectra. This figure shows that by adding the methylating agent, the differences in the raw spectra become more apparent.
figure shows that by adding the methylating agent, the differences in the raw spectra become more apparent. The *Bacillus cereus* is classified separately from the *Listeria innocua* and *Listeria seeligeri*. The Listeria samples are not linearly separated in this figure. Figure 4.7 gives the ion mobility score plot of a sample of *Bacillus cereus* with the addition of 1 µL of TMAH and a sample of *Listeria innocua* with the addition of 1 µL of TMAH to confirm results obtained in Figure 4.6. The analyses were conducted isothermally at 300 °C. This figure encompasses 56.0% of the cumulative variance in the data set in the first two principal components. The *Bacillus cereus* and *Listeria innocua* are separated.

Figure 4.8 gives the ion mobility spectral score plots of a sample of the *Listeria innocua* with 1 µL of TMAH and a sample of the *Listeria seeligeri* with 1 µL of TMAH. The analyses were conducted isothermally at 300 °C. When investigated without combining the *Bacillus cereus* data set to the run, there is a small section where the two Listeria overlap. It is believed that this is a result of their peak in common at a $K_0$ of 1.98 cm²V⁻¹s⁻¹. However, the differences in the two Listeria are more evident in this figure.

Results obtained to this point were promising, but better classification was achieved by adding an important step. Instead of using the raw spectra for PCA analysis, SIMPLISMA extracted spectra were utilized. The SIMPLISMA extracted spectra provided several important features that could not be utilized with the raw spectra. First of all, all evidence of the nicotinamide and TMAH peaks could be removed from the data used for PCA analysis. In raw spectra, the presence of these peaks could not be completely removed. Only parts of the data set, where the nicotinamide or TMAH were
Figure 4.7 Ion mobility spectral score plot showing 56.0% of the cumulative variance between the first two principal components of bacteria after THM. (B) *Bacillus cereus*, 335 raw spectra; (I) *Listeria innocua*, 323 raw spectra. The *Bacillus cereus* and *Listeria innocua* are separated.
Figure 4.8 Ion mobility spectral score plot showing 25.8% of the cumulative variance between the first two principal components of bacteria after THM. (I) Listeria innocua, 300 raw spectra; (S) Listeria seeligeri, 231 raw spectra. When investigated without combining the Bacillus cereus data set to the run, there is a small section where the two Listeria overlap. It is believed that this is a result of their peak in common at a $K_0$ of 1.98 cm$^2$V$^{-1}$s$^{-1}$.
the only peaks present, could be removed. Also, the two peaks at reduced mobilities of 1.71 and 1.63 cm²V⁻¹s⁻¹, termed the bacterial components, which were present in all bacteria studied, could also be removed. Therefore, information specific to each bacterium could be utilized for identification and classification. Table 4.1 provides the reduced mobilities of the peaks present in the SIMPLISMA extracted spectra utilized in PCA studies.

Figure 4.9 gives the SIMPLISMA extracted spectra for a sample of *Bacillus cereus* with the addition of 1 µL of TMAH. This analysis was conducted isothermally at 300 °C. From this figure, it is obvious that spectra are complex with multiple peaks and possible overlapping peaks. By utilizing SIMPLISMA, peaks are grouped according to their thermal profiles. Figure 4.10 and Figure 4.11 give SIMPLISMA extracted spectra for sample of *Listeria innocua* and *Listeria seeligeri* with the addition of 1 µL of TMAH, respectively. These analyses were conducted isothermally at 300 °C. Differences in spectral features can be visualized by comparing these three figures. These differences allow the detection and identification of the different bacteria.

The pure variables corresponding to the *Bacillus cereus*, *Listeria innocua*, and *Listeria seeligeri* were studied by PCA. Figure 4.12 gives the ion mobility spectral score plot of the THM bacteria SIMPLISMA results. Each experiment consisted of placing bacterial whole cells on an IMS filter disk, adding 1 µL of TMAH, and analyzing by thermal desorption IMS. Each sample was also taken from a different plate to ensure reproducible results. Over seventy-three percent of the cumulative variance is provided by the first two principal components. From this figure, the three different bacteria are
Figure 4.9 SIMPLISLMA extracted spectra of THM *Bacillus cereus* whole cells. This analysis was conducted isothermally at 300 °C. Components are ordered by SIMPLISMA purity value.
Figure 4.10  SIMPLISLMA extracted spectra of THM Listeria innocua whole cells.

This analysis was conducted isothermally at 300 °C. Components are ordered by SIMPLISMA purity value.
Figure 4.11 SIMPLISLMA extracted spectra of THM *Listeria seeligeri* whole cells. This analysis was conducted isothermally at 300 °C. Components are ordered by SIMPLISMA purity value.
Figure 4.12 Ion mobility spectral scores showing 73.4% of the cumulative variance between the first two principal components of THM bacteria SIMPLISMA results. (B) *Bacillus cereus*, 5 experiments; (I) *Listeria innocua*, 5 experiments; (S) *Listeria seeligeri*, 5 experiments. Each experiment consisted of placing bacterial whole cells on an IMS filter disk, adding 1 μL of TMAH, and analyzing by thermal desorption IMS. Each sample of bacteria was also taken from a different plate to ensure reproducible results. From this figure, the three different bacteria are nicely clustered.
nicely clustered. The differences in the SIMPLISMA spectra provided enough information to distinguish between the different bacteria.

After receiving the proper certification to cultivate biosafety level 2 bacteria, *Listeria monocytogenes* was investigated. The SIMPLISMA extracted spectra for a sample of *Listeria monocytogenes* with the addition of 1 µL of TMAH is given in Figure 4.13. *Listeria monocytogenes* has three characteristic peaks at reduced mobilities of 1.54, 1.47, and 1.41 cm²V⁻¹s⁻¹. These reduced mobilities can be compared to reduced mobilities of peaks from the other bacteria investigated. This information is provided in Table 4.1. Samples of THM *Bacillus cereus*, *Listeria innocua*, and *Listeria seeligeri* were also rerun on the spectrometer when the *Listeria monocytogenes* experiments were conducted. Differences in these spectra allowed identification of the different bacteria. The pure variables corresponding to the *Bacillus cereus*, *Listeria innocua*, *Listeria seeligeri*, and *Listeria monocytogenes* were studied by PCA. Figure 4.14 gives the ion mobility spectral score plot of the THM bacteria SIMPLISMA results. Each experiment consisted of placing bacterial whole cells on an IMS filter disk, adding 1 µL of TMAH, and analyzing by thermal desorption IMS. Each sample was also taken from a different plate to ensure reproducible results. Over sixty percent of the cumulative variance is provided by the first two principal components. From this figure, the four bacteria are clustered.

4.4. Discussion and Conclusion for THM-IMS

By utilizing thermal hydrolysis/methylation-ion mobility spectrometry, different bacteria could be detected, identified, and classified by differences in their FAME
**Figure 4.13** SIMPLISLMA extracted spectra of THM *Listeria monocytogenes* whole cells. This analysis was conducted isothermally at 300 °C. Components are ordered by SIMPLISMA purity value.
Figure 4.14  Ion mobility spectral scores showing 60.2% of the cumulative variance between the first two principal components of THM bacteria SIMPLISMA results. (B) *Bacillus cereus*, 5 experiments; (I) *Listeria innocua*, 5 experiments; (S) *Listeria seeligeri*, 5 experiments; (M) *Listeria monocytogenes*, 5 experiments. Each experiment consisted of placing bacterial whole cells on an IMS filter disk, adding 1 µL of TMAH, and analyzing by thermal desorption IMS. Each sample of bacteria was also taken from a different plate to ensure reproducible results. From this figure, the bacteria are clustered.
profiles. Bacteria specimens in the same species could be differentiated along with bacteria in different species. The THM produced FAMEs that could be detected by IMS. Resulting peaks could be identified by their reduced mobilities, and differences in the resulting spectra were exploited by PCA to classify the bacteria. By coupling SIMPLISMA results with PCA, better classification of the bacteria was achieved.

This method appears promising as a screening method for the detection of bacteria on food with analysis times of approximately twenty seconds. More work must be conducted before definitive conclusions on the applicability of this method can be made. More species of bacteria need to be investigated. Also, laboratory equipment is needed to determine detection limits possible by this method. Finally, growing the bacteria on food to determine if detection is possible in non-controlled growing conditions would be necessary before stating conclusively the applicability of this method.
Chapter 5. Conclusions and Future Work

In Chapter 1, a brief overview of IMS and SIMPLISMA was given, and the motivations of the research projects were stated. Fast screening methods are necessary for solving forensic problems, and many forensic problems can be solved without using hyphenated systems which are more time consuming and complex. Coupling SIMPLISMA with IMS allowed temporal changes to be exploited instead of lost which could occur when averaging.

In Chapter 2, coupling a temperature ramped thermal desorption step to the ion mobility measurement allowed components to be separated based on their vapor pressure. This ramped thermal desorption allowed separation of components in a mixture; therefore, interferents could be separated from analytes of interest. These changes were then modeled by SIMPLISMA. This system would reduce the occurrence of false positive or false negative responses that could occur in an airport.

Further research can be done to extend the number of explosives and interferents investigated. This would include incorporating interferents that actually cause false positives and false negatives in the airport setting. These interferents could include perfumes, colognes, lotions, or even medicines such as cough syrup. The development of a more versatile heater would also be beneficial. The heater utilized could only accurately increase at a rate of 4 °C/s. For a twenty second run, that encompassed an 80 °C range which would be sufficient. However, to accomplish the six second time limit the FAA imposes on all explosive monitoring systems, the range is reduced to 24 °C at its highest operating capabilities. The system has the capabilities of collecting up to fifty
spectra per second. Slight changes as the temperature of the desorber heater is increased could easily be captured and used to determine the components of the mixture. This method can also be applied in positive ion mode for the separation of narcotics from interferents. This would help to solve problems such as identifying methamphetamine or other drugs in the presence of nicotine in drug labs.47

In Chapter 3, coupling SPE to IMS showed the benefits afforded by combining these two methods. Detection of trace levels of some common explosives in water was achieved at lower detection limits than IMS could achieve solely. Also, detection of illegal narcotics and their metabolites in urine was possible at the SAMSHA cut-off limits for both the initial screen and confirmation test. The presence of several common adulterants did not affect the detection of the analytes by SPE-IMS. Both instances show the ability of IMS when coupled to SPE to detect analytes in liquid matrices at very low concentrations. Also, interferents were removed and did not hinder the detection of the analyte of interest.

Future research would include extending the types of explosives and narcotics studied along with the different metabolites of the narcotics. Also, the addition of possible derivatizing agents that could be utilized to detect analytes that normally do not produce IMS spectra should be investigated. Automating this system is a very important next step. Automating the system would allow large numbers of samples to be run in relatively short periods of time. Automation is also a very important aspect when handling biological fluids such as urine where contamination is possible. For the detection of narcotics, the use of prescription drugs would have to be investigated to
determine how the results would affect the detection of the narcotics or their metabolites. This could be accomplished by using urine specimens from individuals taking prescription drugs. Also, studying real world samples instead of spiked samples would be important for both the explosives and narcotics SPE-IMS projects to determine actual applicability. Also, using the SPE-IMS to detect trace components in air should be investigated. This could be applied to long-term monitoring which would correspond to long-term exposure. One examples of where this would be applicable is detecting TNT vapors in air in the workplace during an eight hour workday or a forty hour work week. Both the Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) have set maximum levels of exposure to TNT\textsuperscript{57} and RDX\textsuperscript{58} in workplace air. By developing a system where room air could be directed through the SPE disk, the exposure to such compounds could be investigated.

In Chapter 4, combining a derivatizing step to the thermal desorption IMS allowed for the detection and identification of different types of bacteria. This was accomplished by using a byproduct of the bacteria, lipids. These lipids were converted to fatty acid methyl esters, and the fatty acid methyl esters were detected by the IMS. Extending both the types of bacteria investigated and the types of growth media used are very important to determine if this is a universal method that can be applied in the food industry.

Further research in this method would be dependent on several factors. First of all, determining limits of detection would be necessary. To accomplish this, hardware
not currently available, such as cell counters and microscopes, would be needed. Also, an incubator would be beneficial in allowing accurate time data to be collected.

Although all samples were collected 24 hours after inoculation, the rate of growth of the samples differed on the plates. In a controlled environment, the growth of samples could be optimized. Finally, the investigation of bacteria on food would be important. Determining if growth media caused an effect on the detection and identification of the bacteria would be important. If bacteria grown on different substances produced different lipids and therefore different results, it would be important to know and test bacteria on these different substances to produce profiles. Also, TMAH was the only derivatizing agent utilized in this study. Looking at different methylating agents would provide a comparison on the efficiency of the methylating agent.

It is important to exploit the advantages inherent in IMS. Some of these very important properties include its portability, its ability to produce rapid results, its sensitivity, and its real-time monitoring capabilities. Some of these advantages which make IMS an ideal screening method are lost when more complex systems are used to solve problems.
References


(64) Walsh, M. E.; Ranney, T. Determination of nitroaromatic, nitramine, and nitrate ester explosives in water using solid-phase extraction and gas chromatography


Appendix A: Publications


3) Buxton, T.L.; Harrington, P.B. Trace explosive detection in aqueous samples by solid phase extraction ion mobility spectrometry (SPE-IMS). *Appl. Spectrosc.* **2002**. In revision.

4) Buxton, T.L.; Harrington, P.B. The detection of illegal narcotics and their metabolites in urine by solid phase extraction ion mobility spectrometry (SPE-IMS). **2002**. To be submitted.

Appendix B: Presentations


2) **Buxton, T.L.; Harrington, P.B.** “Modified ion mobility spectrometer for enhanced selectivity”, oral presentation at the 26th Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies, Vancouver, BC, Canada, October 28, 1999, 705.


4) **Buxton, T.L.; Harrington, P.B.** “Rapid classification of bacteria by ion mobility spectrometry” oral presentation at the 52nd Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy, New Orleans, LA, March 6, 2001, 384