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DESIGN AND IMPLEMENTATION OF GAS CHROMATOGRAPHY/MASS SPECTROMETRY METHODOLOGIES FOR THE ANALYSIS OF THERMALLY LABILE DRUGS AND EXPLOSIVES

For the degree of Master of Science

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DESIGN AND IMPLEMENTATION OF GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) METHODOLOGIES FOR THE ANALYSIS OF THERMALLY LABILE DRUGS AND EXPLOSIVES

A Thesis

Submitted to the Faculty

of

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Jordan R. Ash

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For my family and friends who always encourage me to strive to be better than I am.

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ABSTRACT

Ash, Jordan R. M.S., Purdue University, December 2016. Design and Implementation of Gas Chromatography/Mass Spectrometry (GC/MS) Methodologies for the Analysis of Thermally Labile Drugs and Explosives. Major Professor: John V. Goodpaster.

Gas Chromatography/Mass Spectrometry (GC/MS) is an analytical technique that sees frequent use in labs across the world. It is also one of the most common instruments found in forensic science laboratories. This technique can efficiently and accurately separate and identify a broad range of compounds that may be present in evidence submitted for analysis. In this work, the versatility of this instrument was applied to new methodologies for the detection of explosives and illicit drugs.

The analysis of explosives by GC/MS is common but can be problematic. The thermally sensitive nature of some explosives can cause them to degrade when introduced to the high temperatures of a GC/MS inlet. This project looked at the design and implementation of a way to separate and detect a variety of nitrate ester explosives in a short amount of time. In addition to this, a new technique known as Total Vaporization-Solid Phase Microextraction (TV-SPME) was utilized as a pre concentration technique. The parameters for TV-SPME were statistically optimized for a

low level of detection. The combination of these areas allowed for the separation of ethylene glycol dinitrate, nitroglycerin, erythritol tetranitrate, and pentaerythritol tetranitrate with a detection limit as low as 50 parts per trillion (ppt). Degradation products such as 1-mononitroglycerin, 1-3-dinitroglycerin, and 2-mononitroglycerin were also successfully identified.

The problem of thermally labile compounds extends to the world of illicit drugs. In the second project, several derivatization schemes were developed for common controlled substances. N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was used for silylation, trifluoroacetic anhydride (TFAA) was sued for acylation, and (N,N-Dimethylformamide dimethyl acetal (DMF-DMA) for alkylation. Three different compound classes totaling 15 different drugs were investigated. N,N-Dimethylformamide dimethyl acetal (DMF-DMA) is presented as a novel way of derivatizing several drugs of interest. Primary amines and zwitterions were derivatized with this reagent to much success, specifically: amphetamine, 2-(4-Iodo-2,5-dimethoxyphenyl)ethan-1-amine (2C-I), pregabalin, and gabapentin.

CHAPTER 1. DESIGN AND OPTIMIZATION OF A GAS CHROMATOGRAPHY/MASS SPECTROMETRY METHOD FOR THE SEPARATION AND IDENTIFICATION OF NITRATE ESTERS

1.1 Introduction

The utilization of Improvised Explosive Devices (IED's) to perform acts of terrorism has been well documented[1]. These devices vary widely in their size, explosive potential, and components. For example, the Olympic park bombing in 1996 used smaller pipe bomb devices that could be concealed and utilized dynamite as the explosive[1]. The Boston marathon bombing used a slightly larger device than the pipe bombs used in the Olympic park bombing. A pressure cooker in a backpack using a pyrotechnic composition as the accelerant was used. In both of the instances the devices were easily carried and hidden from view. This is not always the case. The bombing of the World Trade Center in 1993 used a van that was loaded with urea nitrate as the main charge. The broad range of explosives and devices that can be used can cause issues for forensic scientists, particularly in a post-blast investigation. After a device is set off it consumes most explosive that was present, as well as spreads the components of the device

around the blast area[2]. Hence, there is a need for methods to detect explosives at low concentrations and from various substrates.

Depending on the suspected explosive filler that was used in a device, the type of analysis will differ. The Environmental Protection Agency has standard procedures for the analysis of organic explosives using both High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC). HPLC is typically used with an Ultraviolet (UV) detector[3], whereas a GC will typically use an Electron Capture Detector (ECD). Both of these methods are sensitive and relatively selective[4]. Specificity is a necessity due to the vast range of explosive fillers that could be used. Specificity becomes of particular concern with nitrate esters. These are compounds that are not very active in the traditional UV range and cannot be differentiated using an ECD[2, 5]. This then suggests the need for a sufficient separation technique prior to detection using a mass spectrometer (MS). Both HPLC and GC adequately separate a large range of explosives of interest, however; HPLC is better suited to some that are more thermally unstable[6].

Using a Mass Spectrometer (MS) coupled to a GC can add specificity as well as sensitivity to analysis, specifically using a MS in the Negative Ion Chemical Ionization (NICI) mode[4]. Like an ECD, NICI is particularly sensitive to electronegative and/or electron withdrawing functional groups like nitrated explosives[7]. This can further increase the sensitivity of the experiment. CI is also considered a “softer” ionization technique than traditional Electron Impact (EI) MS. This is due to the creation of lower energy electrons by the reagent gas in chemical ionization as compared to the 70 eV electrons produced in an EI source. Because of this there is less fragmentation. The

appearance of CI spectra depends heavily on the conditions used; hence database/library searches are not typically available. However, with nitrate esters, they will all fragment reliably into a m/z 46 (NO_2^-) and a m/z 62 (NO_3^-)[8]. Because of this, NICI can be used reliably in the analysis of nitrate esters. Utilizing a NICI MS increases the sensitivity, but it does not eliminate the problems with thermal stability or identical fragmentation. Thus the goal of this project was to find a method that is able to adequately separate various nitrate esters while preventing thermal degradation at concentrations commensurate with residue recovered from exploded devices.

By adjusting the way that the sample is introduced to the GC, the analyst can increase sensitivity and potentially decrease thermal degradation. The reduction of thermal degradation is based primarily on the temperatures that the sample encounters prior to the oven program on the GC. By using various forms of sampling and pre-concentration, an analyst can attempt to increase the sensitivity of the instrument. Liquid injection, Solid Phase Microextraction (SPME), and traditional headspace are all common ways to introduce a sample to a GC and are all used in the analysis of explosives[4].

Traditional headspace has been used in the analysis of intact explosives due to the volatility of the analytes[9]. Specifically, headspace SPME has been used to determine the volatiles present in explosives such as C-4 and Semtex for use in determining the profile that an explosive sensing canine is sensitive to[10]. A vial containing a small amount of intact explosive is sampled at room temperature for a sufficient amount of time to allow the volatiles present to adsorb onto a SPME fiber prior to being desorbed in the

inlet of a GC/MS. However, due to the nature of this sampling technique the space above the sample must be relatively saturated with analyte and is thus not agreeable with the low concentrations normally found on device residue.

SPME is a pre-concentration technique where a sample is adsorbed onto a coated fiber and then desorbed in the inlet[11]. SPME can be done in headspace or immersion modes. Headspace SPME would be where the vial is partially filled with an amount of liquid and then the fiber is exposed to the headspace until equilibrium occurs between the fiber, the gaseous phase above the sample, and the liquid sample[10, 12]. Immersion SPME is a 2-phase system where the fiber is completely immersed into a liquid so that equilibration only must occur between the fiber and the liquid sample. Both of these techniques have been applied to a variety of different analytes and due to the nature of being a pre-concentration technique have an increase in sensitivity over liquid injection. However, both of these SPME techniques have their downsides. Headspace SPME has to rely on the analyte being able to escape the matrix that it is in and reach the headspace on the vial. This can be of concern depending on the type of sample that is being analyzed. Equilibrium present in the sampling vial is explained by an equation specific to the type of sampling. The equations for simple headspace, headspace SPME, and immersion SPME are explained below in equations 1-1, 1-2, and 1-3 respectively[11].

$$C_G = \frac{C_O}{(K + \beta)} \qquad \text{(Equation 1-1)}$$

C_G is the concentration of analyte present in the headspace, C_O is the concentration of the sample prior to analysis, K is the partition coefficient of a given compound between the liquid and gaseous phase, and β is the phase ratio. This can be helped by using immersion SPME, as previously stated. However immersion SPME does not fully eliminate potential matrix effects, but it does eliminate the need for the analyte to partition from the liquid phase to the gaseous phase prior to adsorption. However, a downside to immersion SPME is the decrease in lifespan of SPME fibers. With immersion SPME and headspace SPME the fiber must also be taken into account as well as the mass diffusion of the sample to the fiber and the phases that are involved. This relationship for immersion SPME is described by the following:

$$n = \frac{K_{fs} V_s V_f C_0}{K_{fs} V_f + V_s} \approx K_{fs} V_f C_0 \qquad \text{(Equation 1-2)}$$

Where n is the mass of analyte on the fiber, K_{fs} is the distribution coefficient between the sample and the fiber, V_s is the volume of sample, V_f is the volume of the fiber coating, and C_0 is the initial concentration of analyte in the sample.

$$n = \frac{K_{fh} K_{hs} V_f C_0 V_s}{K_{fh} K_{hs} V_s + K_{hs} V_h + V_s} \qquad \text{(Equation 1-3)}$$

Where n is mass of analyte extracted; K_{fh} is the coating/gas distribution constant; K_{hs} is the gas/sample matrix distribution constant; V_f, V_h, and V_s are the volumes of the coating, the headspace, and the matrix respectively; and C_0 is the original concentration of sample in the sample.

A new technique that has been pioneered by the Goodpaster group takes some of the benefits of simple headspace and immersion SPME and combines them together. Eliminating the matrix in traditional headspace has been done by utilizing total vaporization headspace sampling. Total vaporization headspace is a technique that takes a small amount of sample and completely vaporizes it inside of the sample vial, thus eliminating any effects that various matrices may have on preventing the analyte from partitioning into the gaseous phase[13-14]. This matrix eliminating practice has been coupled with the increase in sensitivity that SPME offers to give rise to a new sampling technique known as Total Vaporization Solid Phase Microextraction (TV-SPME)[15]. With TV-SPME, like total vaporization headspace sampling, a small amount of sample is completely vaporized prior to sampling. The amount of sample that can be analyzed is explained via the following equation:

$$V_s = \frac{\left(10^{A-\frac{B}{T+C}}\right)V}{RT}\left(\frac{M}{\rho}\right) \qquad \text{(Equation 1-4)}$$

where V_s is the volume of the sample in the vial (mL), A, B, and C terms are the Antoine constants that describe the vapor pressure of the solvent being used at the incubation

temperature T, V is the volume of the vial (L), M is the molar mass of the solvent (g/mol), R is the ideal gas constant (8.3145 X 10^{-2} L bar/K mol), and ρ is the density of the solvent at the temperature it was placed into the vial (e.g. room temperature) (g/mL). TV-SPME has been used in quantitation of explosive residue and on nicotine present in hair[15-17]. TV-SPME is most analogous to immersion SPME in that it is only a 2-phase system. An illustration of the TV-SPME process can be seen in Figure 1-1.

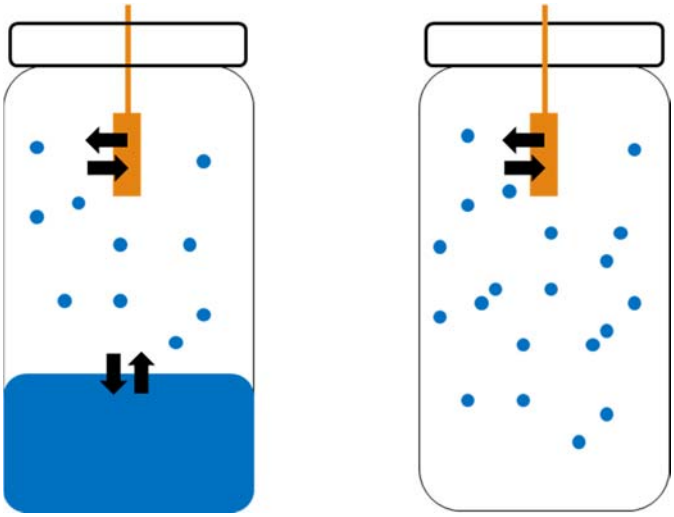


Figure 1-1: Illustration of TV-SPME (right) compared to headspace SPME (left)

By completely vaporizing the sample prior to equilibration the matrix is longer present to prevent the analyte from reaching the gaseous phase. This also allows for the added benefit of eliminating the need for a sample clean-up step.

EPA Method 8095, Explosives by Gas Chromatography, references EPA Method 3535, Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC), for extraction procedures of explosives from soil[18-19]. When a device goes off the ground around the device will now contain residue from the explosive filler. This soil is

then collected, extracted and filtered prior to analysis[18]. With TV-SPME the filtration step may not be necessary as only volatile compounds will enter the gaseous phase to adsorb onto the SPME fiber, thus any potential contamination will remain on the bottom of the vial. Also, matrix effects are eliminated as the entire matrix is dispersed upon total vaporization.

1.2 Materials and Methods

1.2.1 Materials

Ethylene glycol dinitrate (EGDN) (1mg/mL), nitroglycerin (NG) (1mg/mL), and pentaerythritol tetranitrate (PETN) (1mg/mL) were all purchased in methanolic solution from Restek. Dichloromethane, HPLC grade, was purchased from Fisher Scientific. All SPME fibers were purchased from Sigma Aldrich. Erythritol tetranitrate (ETN) in acetonitrile (1mg/ml), 1-monitroglycerin in 50:50 methanol acetonitrile (1mg/mL), 1-3-dinitroglycerin in 50:50 methanol acetonitrile (1mg/mL), and 2-mononitroglycerin (1mg/mL) in 50:50 methanol acetonitrile were purchased from AccuStandard. SPME vials and caps were purchased from Gerstel and liquid caps and vials were purchased from Fisher Scientific.

1.2.1.1 TV-SPME

Total Vaporization-Solid Phase Microextraction (TV-SPME) was used as a pre-concentration technique prior to GC/MS analysis. TV-SPME is analogous to liquid immersion SPME in that both are two-phase systems [15]. In TV-SPME, a small aliquot is placed in a vial and heated until the sample completely vaporizes. A fiber is then introduced to the vial and the sample is adsorbed onto the fiber coating. This method has been shown to have increased sensitivity over traditional liquid injection[15, 17]. Due to the sample being completely vaporized there is no need for a filtration or clean-up step prior to analysis as any material that is insoluble or non-volatile will stay on the bottom of the vial. The sample volume that can be analyzed using this method is directly related to the properties of the solvent that contains the analyte as well as the temperature at which the sample is being held at prior to extraction. This relationship was previously explained in Equation 1-4.

1.2.2 Instrumental

A Thermo Trace GC Ultra coupled to a Thermo DSQ II mass spectrometer (MS) was used for all analysis, along with a Thermo TriPlus autosampler. Utilizing the incubation station for the autosampler, samples were heated to 60°C for 1 minute prior to the introduction of the polydimethylsiloxane (PDMS) fiber for 5 minutes. The fiber was then introduced to a programmed temperature vaporization (PTV) inlet for 1

minute. The inlet had a temperature program of 40°C ramped at 3°C/second to 160°C, a hold for 0.3 minutes, ramped again at 3°C/second to 220°C, and a final hold of 1 minute. For liquid injections the same inlet temperature ramp was used, but without a hold at 160°C. The column used was a Zebron ZB5-MS with dimensions of 10m x 0.18mm x 0.18µm. Helium carrier gas was utilized at a flow rate of 2.5 mL/min. The oven temperature program started at 40°C for 1 minute and was then ramped at 40°C/min to 300°C and held there for 1 minute. Post desorption, the fiber was conditioned offline at 240°C for 5 minutes. The mass transfer line into the MS was set to 250°C and the source temperature was set to 200°C. The MS was in the negative ion chemical ionization (NICI) mode using a methane reagent gas at a flow rate of 1.3 mL/min. Selected ion monitoring was used and set at m/z 46 (NO_2^-) and m/z 62 (NO_3^-) for detection of all nitrate esters. The total scan time was 0.24 seconds with a dwell time of 100 milliseconds (ms).

1.2.3 Optimization

In addition to the many parameters that are involved in a traditional liquid injection GC/MS method, TV-SPME introduces a number of different variables[15, 17]. An optimization was done for several of these parameters, including desorption temperature (160-220°C), desorption time (1-5 min), incubation temperature (40-120°C), and extraction time (5-30 min). The variables were tested simultaneously utilizing a response surface methodology (RSM) with a face centered central composite design

(CCD), utilizing Minitab 17 Statistical Software. This is a statistical method that allows for the optimization of a response while varying multiple parameters. A second order RSM was used and is explained via the following equation:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum\sum_{i<j} \beta_{ij} x_i x_j + \epsilon \qquad \text{(Equation 1-5)}$$

Where y is the response, β_0 is a constant, k is the number of variable tested, β_i is the linear term coefficient, x_i is the linear variable, β_{ii} is the square term coefficient, x_i^2 is the square term variable, β_{ij} is the cross term coefficient, $x_i x_j$ is the cross term variable, and ϵ is the error in response[17].

This method of statistical optimization has been applied to TV-SPME previously with much success[8, 15, 17]. A total of 31 experimental runs was determined by an α of 1 and an η_c of 7. Where α is the distance the axial points are from the center and η_c is the number of center points [(2)].

1.2.4 Sensitivity Comparison

The sensitivity of TV-SPME and liquid injection for EGDN was compared, ranging in concentration from 0.05 pg/mL to 1 µL/mL. The same parameters were used for both the TV-SPME samples and the liquid injection samples, with the exception of liquid injection not having an isothermal hold during the ramp of the PTV inlet during injection.

A hold was necessary in TV-SPME due to the interaction of the analyte with the fiber. An injection volume of 1 µL was used for liquid injection and 70 µL was used for the sample volume of TV-SPME. Both methods used the splitless mode with a splitless time of 1.0 minute.

1.2.5 Flow-Rate Study

The effect that the flow-rate of the carrier gas (helium) had on the chromatography of the analytes of interest was also studied. For this experiment, liquid injections of nitroglycerin at 10 ppm were completed at 1.5 mL/min, 2.0 mL/min, and 2.5 mL/min.

1.2.5.1 PTV vs Isothermal Inlet Temperature

It is well known that nitrate esters will succumb to thermolysis at high temperatures[7, 17]. This process normally occurs quickly in the inlet of a GC. Thus it is important to have a method that has a high enough temperature to ensure vaporization of the analyte, but at the same time limiting the degradation that can occur. A series of studies to determine the effect that inlet temperature has on the detection/degradation of PETN and NG were completed. For PETN, the inlet was programmed to several constant temperatures between 40-180°C in liquid and 80°C-220°C in TV-SPME. The

temperatures were set in 20°C increments resulting in a total of 8 runs for each sampling method. A similar experiment was accomplished for NG; the inlet was held at a constant temperature over the range of 40°C-240°C at 20°C increments for a total of 11 runs using TV-SPME. Additional experiments were done with TV-SPME to determine the effect, if any, that a PTV inlet would have on PETN when compared to an isothermal inlet temperature. The inlet ramp rate was adjusted to 1.5°C/sec, 3°C/ sec, 7.8°C/ sec, and 14.5°C/ sec. In addition to this an isothermal hold of 160°C for 0.3 min was tested. This data was then compared to the previous isothermal runs.

1.2.5.2 Nitroglycerin Inlet Temperature Study

In conjunction with the previous study an experiment was conducted to determine if it was possible to eliminate the known degradation product, dinitroglycerin, of nitroglycerin[17]. This is known to occur at high inlet temperatures. A study was done by increasing the isothermal inlet temperature in 20°C increments from 40°C-280°C. The concentration of NG was held constant at 1ppm. This experiment was conducted in the TV-SPME mode.

1.3 Results and Discussion

1.3.1 Liquid Injection

Initial work was done using TV-SPME, however, it was quickly realized that accurate retention times (RT's) and efficient separation of all four analytes needed to be established prior to continuing with further study into TV-SPME. To this end, the chromatographic parameters that were used in the initial work were taken and adapted to traditional liquid injection. Since liquid injection does not have as many critical variables as TV-SPME: extraction temperature, desorption time, adsorption time, and desorption temperature, it allowed for efficient development of a GC method able to separate and identify the four compounds[15].

The first variable that was examined in liquid injection was the flow rate of the carrier gas. NG was analyzed at a flow rate of 1.5 mL/min, 2.0 mL/min, and 2.5 mL/min. The results of this experiment can be seen in Figure 1-2.

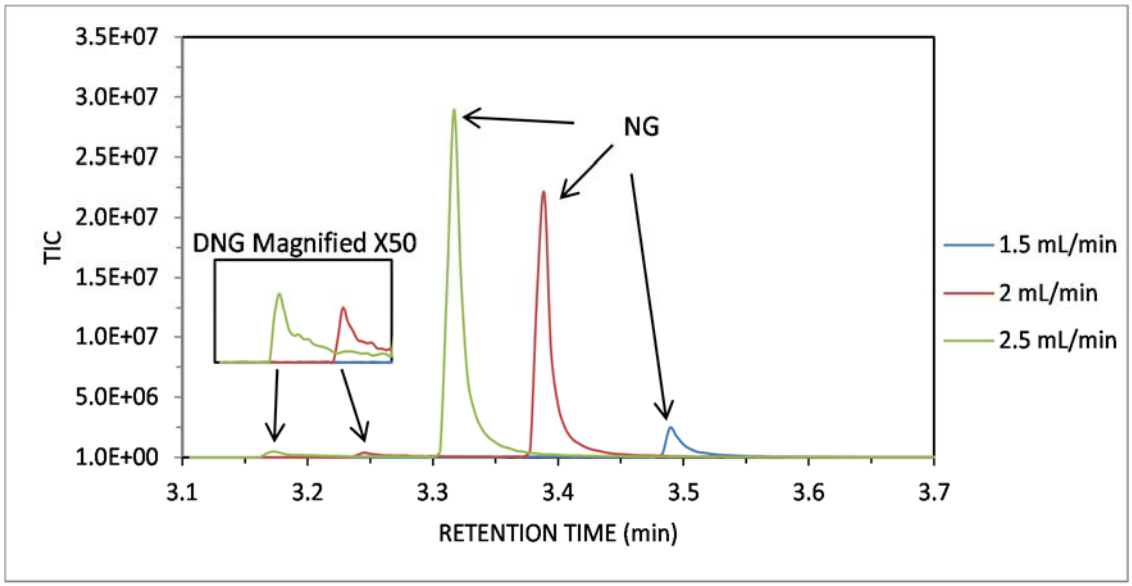


Figure 1-2: Effects of flow rate on the chromatography of NG

*Figure 1-2 *has not been normalized and is a depiction of the true relative abundance of NG and DNG. Peak shape, intensity, and ratio of DNG to NG are all improved as flow rate is increased.*

PETN is thermally labile and was experimentally determined to be the most thermally sensitive of the four primary analytes of interest[20]. Thus additional experimentation was done on PETN to determine appropriate inlet temperature. A series of isothermal inlet temperatures, in 20°C increments, were used from 80°C -180°C. Figure 1-3 and Figure 1-4 depict the results. The data in Figure 1-3 has been normalized for easier visualization.

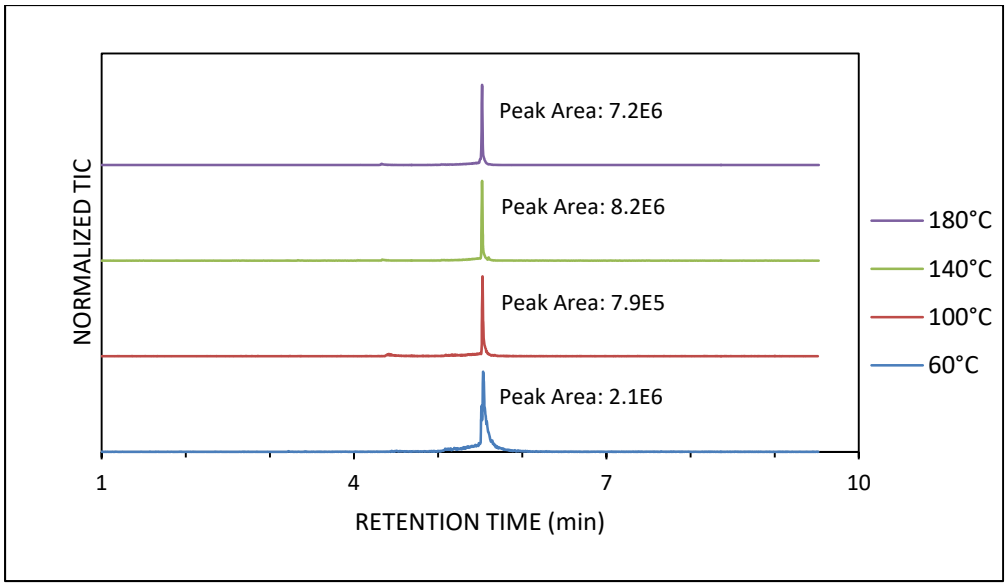


Figure 1-3: Effect of inlet temperature on the chromatography of PETN

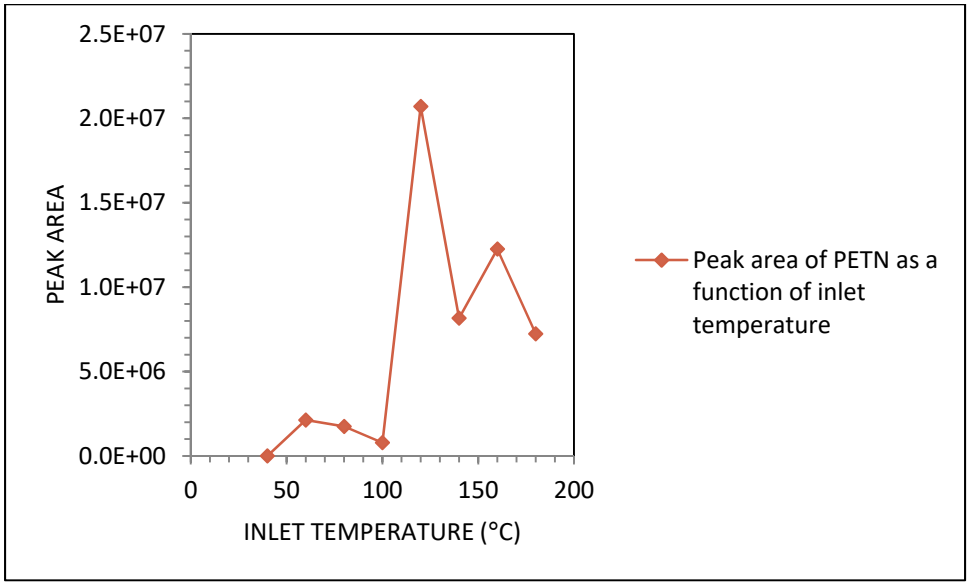


Figure 1-4: Peak area of PETN as inlet temperature increases

Instrument response for PETN increases as the inlet temperature increases until 120°C and begins to fall off at temperatures higher than 160°C. This can be seen in

Figure 1-4. An additional inlet temperature study was done on NG in an attempt to determine at what temperature, if any, degradation of NG to DNG no longer occurs. The results of this can be seen in Figure 1-5. This illustrates that at lower inlet temperatures the degradation product can be eliminated, however at the expense of instrument response to NG. 120°C shows a very small response for DNG, but also a small response for NG. The instrument response for NG is vastly improved at 140-160°C. Based on this, the temperature recommended by the RSM optimization, and the PETN inlet temperature study, the isothermal inlet temperature was established at 160°C.

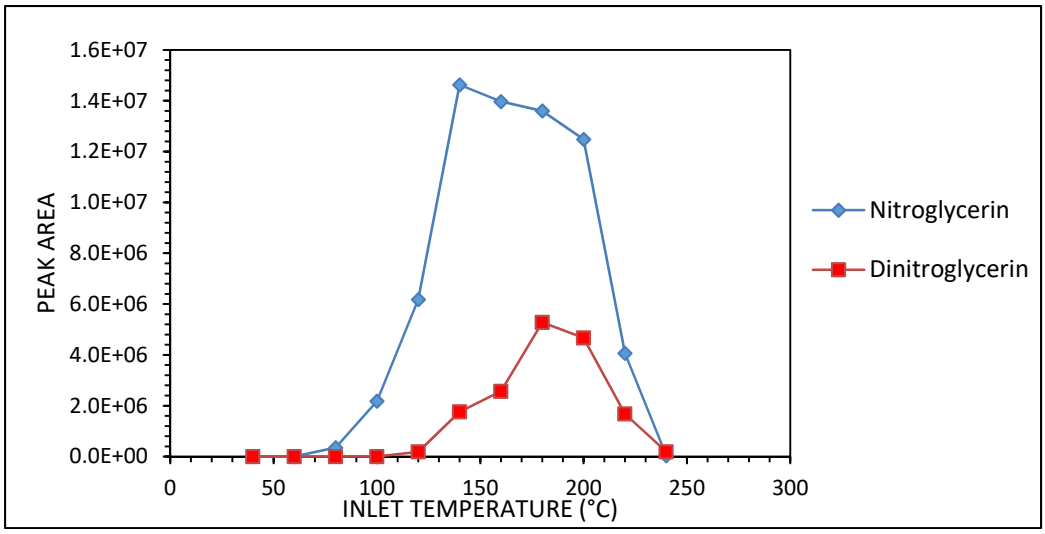


Figure 1-5: Nitroglycerin vs dinitroglycerin peak area at increasing inlet temperatures

Having one method for the identification of various explosives was of the utmost importance throughout experimentation. To ensure that a broad range of nitrate esters with various vaporization temperatures and thermal stabilities could be analyzed, a PTV inlet was utilized to separate four commonly used explosives as well as identification of

three additional compounds that can be found during the analysis of explosives. This separation and identification can be seen in Figure 1-6, each chromatogram has been normalized.

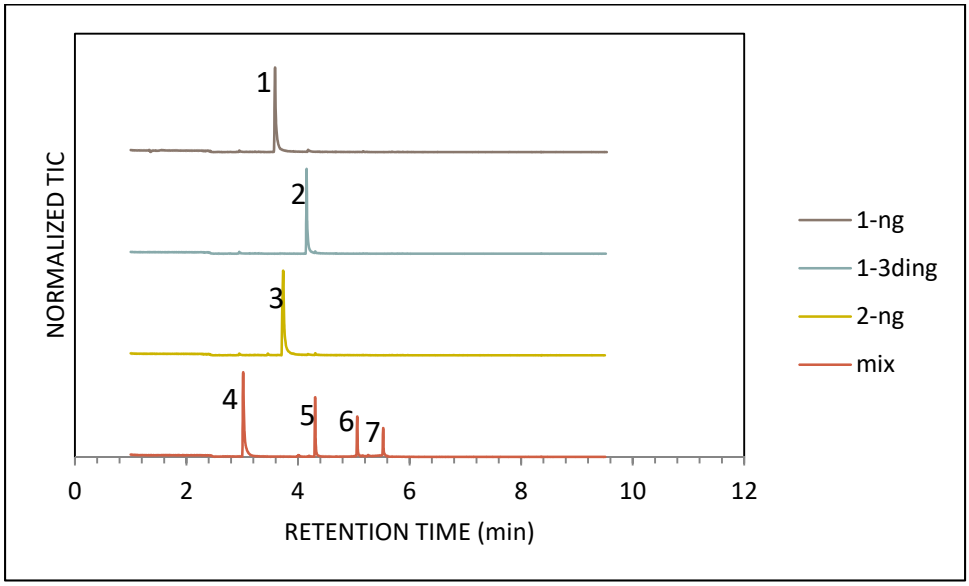


Figure 1-6: Separation of EGDN (1, RT: 3.02), NG (2, RT: 4.31), ETN (3, 5.06), and PETN (4, 5.53) in liquid mode with additional identification of 2-mononitroglycerin (5, RT: 3.73), 1-3-dinitroglycerin (6, RT: 4.15), and 1-mononitroglycerin (7, RT: 3.58)

1.3.2 Optimization of TV-SPME

EGDN, NG, and DNG were the analytes used for optimization. Standards of EGDN and NG were analyzed to confirm retention times of each. DNG is a known degradation product of NG because of hydrolysis of one of the nitro groups[17]. Thus the parameters of the statistical software were set to maximize the signal for EGDN and NG and minimize the signal for DNG. The parameters optimized were as follows: extraction temperature (ETemp), extraction time (ETime), desorption temperature (DTime), and

desorption time (DTime). The results shown in Table 1-1 are the significant parameters for each peak of interest as well as the R^2 value for each. The significant parameters were determined based upon a high F-value (>10) and a low P-value (<0.05). The R^2 value shows how well the data fits the model that was built by the software.

Table 1-1: Significant variables and R^2 values for EGDN, NG, and DNG

Analyte	Significant Variables (linear)	Significant Variables (squared)	Significant Variables (2-way interaction)	R^2
EGDN	ETemp DTime	-	-	0.827
NG	-	ETemp*ETemp Dtemp*DTemp	-	0.958
DNG	DTemp	ETemp*ETemp DTemp*DTemp	-	0.906

Table 1-2 shows the optimization results for each variable individually as well as all variables together. Each calculated optimum also has a desirability score ranging from 0-1. A low desirability score indicates that the optimized parameters given by the software generate a signal that is sub-optimal for each variable taken individually. Variables that result in optimum values at the minimum/maximum for that variable indicate that the true optimum resides outside the range that was tested for that parameter, as the software will only give a value that falls within the range tested. The higher the desirability score, the closer the suggested set of values is to the true optimum for all values. Optimal parameters were produced for EDGN and NG

individually as well as parameters for optimizing the signal for both at the same time and optimizing them both while minimizing the signal for DNG.

Table 1-2: Results for the optimizations of EGDN, NG, and DNG

Analyte	Extraction Temp (°C)	Extraction Time (min)	Desorption Temp (°C)	Desorption Time (min)	Desirability
Max EGDN	40	5	164	5	1.0
Max NG	81	27	184	5	0.982
Max (EGDN + NG)	71	7	180	5	0.912
Max (EGDN + NG) & Min DNG	64	9	160	5	0.814

The data in Table 1-1 indicates that the most important variables are those involving temperature. Thus, based on the optimal values produced for maximizing the signal for EGDN and NG while minimizing the signal for DNG the temperatures were set at 60°C for extraction and 160°C for desorption. 60°C was used for extraction temperature due to the relatively small increase in instrument response as temperature was increased, as well as the increase in degradation products seen as this temperature is increased. Desorption time and extraction time were seen to have minimal effect on the signal of the samples as well as not being correlated to either extraction temperature or desorption temperature. The slope for the instrument response related to either time parameter is shallow, and though response can be increased by increasing

the value of both parameters it was not significant. This method is designed to be utilized in crime laboratories where maximum throughput is a priority, to this end the time parameters were set at their minimum values: 1 min for desorption time and 5 min for extraction time.

1.3.3 Thermal Degradation in TV-SPME

Initial experiments showed that there was severe degradation of PETN and ETN while using TV-SPME. Changing the fiber type from a polar polyethylene glycol (PEG) fiber to a non-polar polydimethylsiloxane (PDMS) fiber allowed for the analytes to desorb from the fiber more easily, helping to prevent thermal degradation. However, thermolysis was still observed, specifically with PETN. An experiment was conducted adjusting the inlet temperature in 20°C increments from 80°C-220°C to determine the best desorption temperature for PETN in TV-SPME. This experiment was able to provide the appropriate isothermal inlet temperature for use with PETN in TV-SPME mode as well as demonstrate the severe thermal degradation that it undergoes. Figure 1-7 shows the results from this, the results have been normalized to 1.

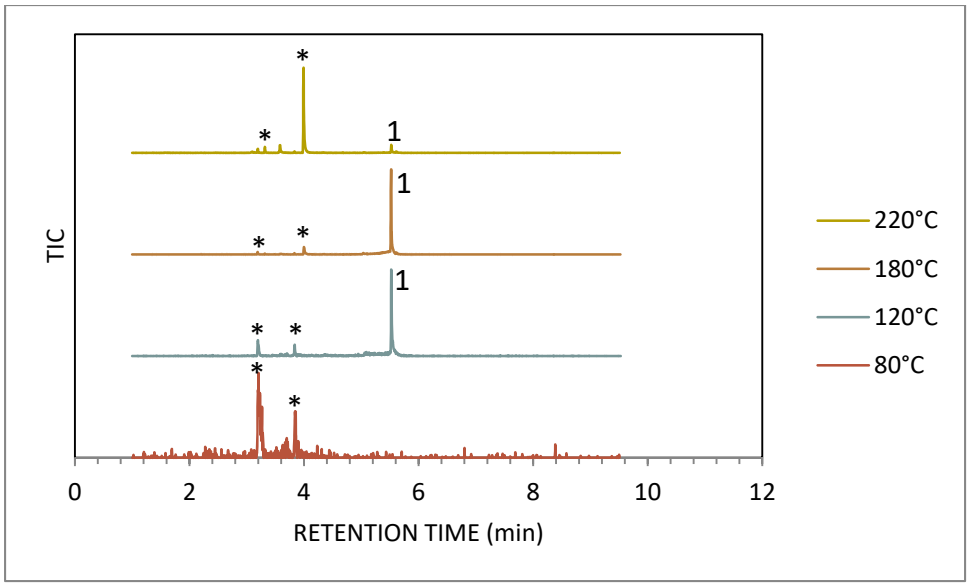


Figure 1-7: Chromatograms of PETN (1) in TV-SPME as inlet temperature increases (*denotes degradation peak)

Figure 1-8 shows a clearer visualization of the thermal degradation that PETN undergoes at higher temperatures. PETN begins to undergo strong degradation around 200°C before being dwarfed by its' degradation products at 220°C.

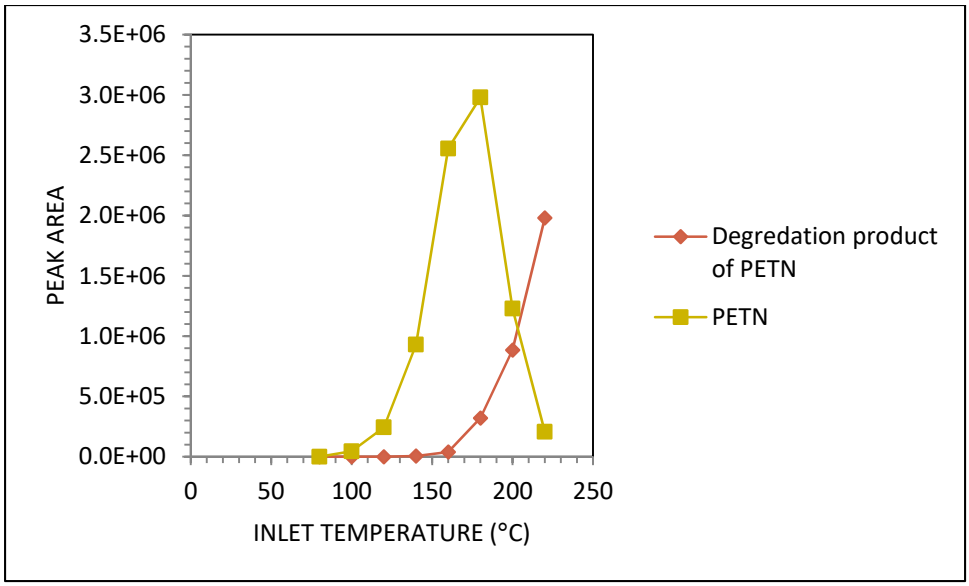


Figure 1-8: Demonstration of the thermal degradation products (*) of PETN (1) formed at high inlet temperatures

Based on this data the inlet temperature for isothermal experiments was set to 160 degrees.

Upon establishing the inlet temperature for TV-SPME the PTV inlet was again utilized. Initial experiments showed that there was severe degradation occurring. Previous experiments showed that if the analyte is not able to easily release from the fiber then it will be exposed to the higher inlet temperatures where thermolysis for that analyte can occur. Thus steps were taken to facilitate desorption at the optimal temperature for each analyte. The temperature ramp speed of the PTV inlet was set to four different speeds: 1.5°C/sec, 3°C/sec, 7.8°C/sec, and 14.5°C/sec. PETN was again the analyte seemingly most sensitive to inlet temperature as well as the analyte that has the most difficulty desorbing off of the fiber at the appropriate temperature when using the

PTV inlet, thus PETN was used for this experiment. The results can be seen in Figure 1-9. A column graph of peak area verses inlet temperature ramp rate for PETN and the primary degradation product of PETN can be seen in Figure 1-10. This shows the thermal liability of PETN as well as the direct relationship that PETN and its primary degradation product have with each other.

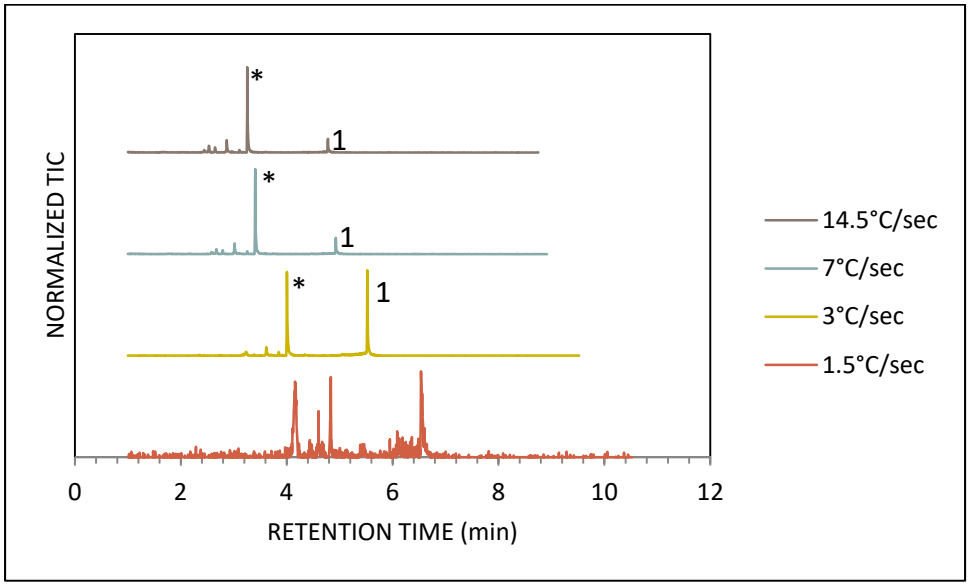


Figure 1-9: Effect that PTV temperature ramp rate has on degradation (*) and peak area of PETN (1)

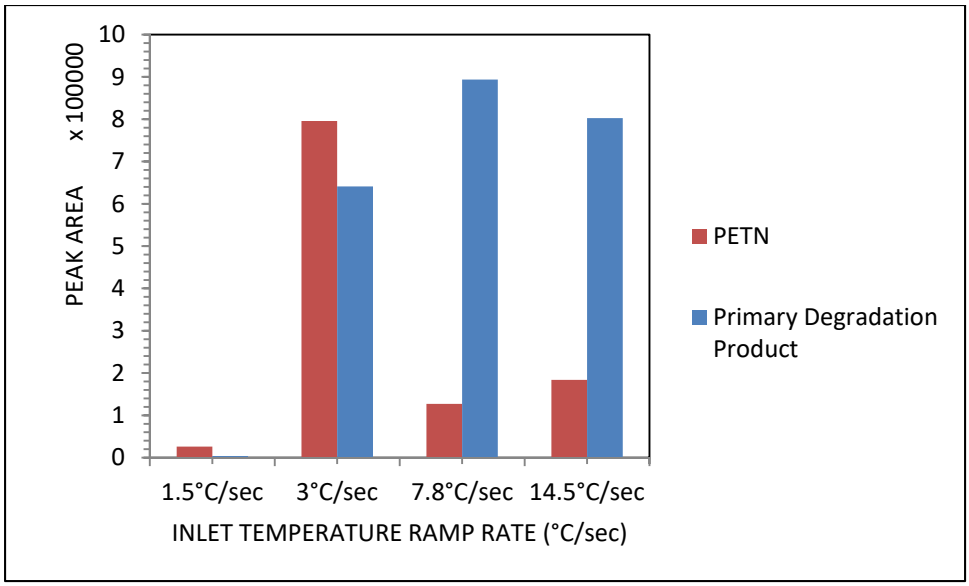


Figure 1-10: Comparison of the peak area of PETN and its primary degradation product as a function of inlet temperature ramp rate in TV-SPME

Although degradation occurs at all temperature ramps, 3°C/sec causes the least amount. Higher ramp rates seem to not allow sufficient time for the analyte to desorb off of the fiber prior to reaching temperatures where degradation occurs. The slowest ramp exhibits full degradation of PETN lending itself to the idea that prolonged exposure to even mild temperatures is enough for analyte loss. In an attempt to further limit the destruction of analyte in the inlet a hold at 160°C for 0.3 min was applied during the course of a 3°C/sec ramp from 40°C to 220°C. The comparison of these two PTV methods can be seen in Figure 1-11. This unconventional temperature progression allowed for desorption of all four key analytes from the fiber, with limited degradation, while maintaining the versatility that a PTV inlet can provide.

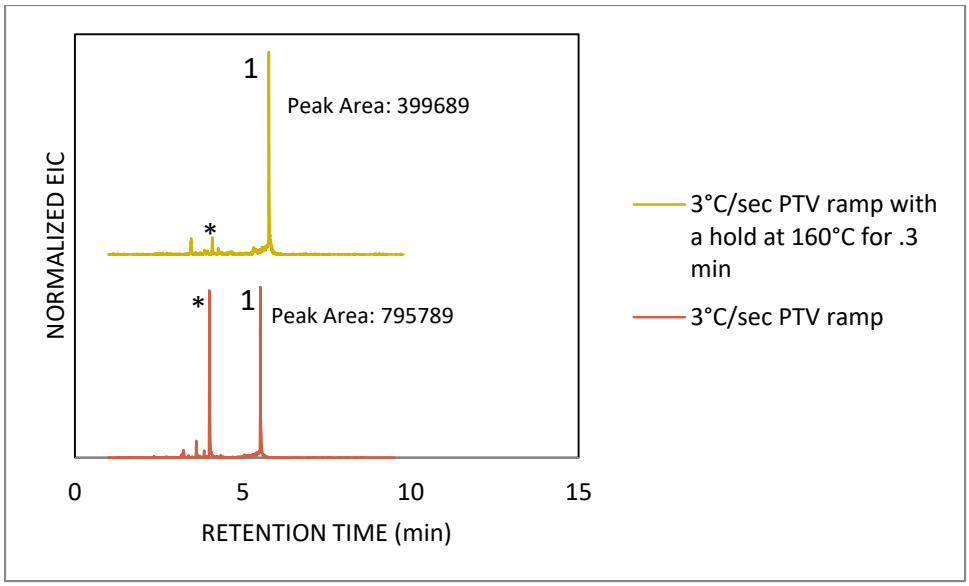


Figure 1-11: Comparison of a PTV inlet ramp for PETN (1) and its primary degradation product (*) with a mid-method hold at 160°C and without

This final method method was then compared to an isothermal inlet at 160°C to confirm the viablitly of the PTV method. Both samples contained EGDN and NG at 1ppm and PETN and ETN at 10 ppm. The intensity of instrument response is at the same order of magnitude for both methods. The results of this are shown in Figure 1-12. The instrument response was not normalized for this data.

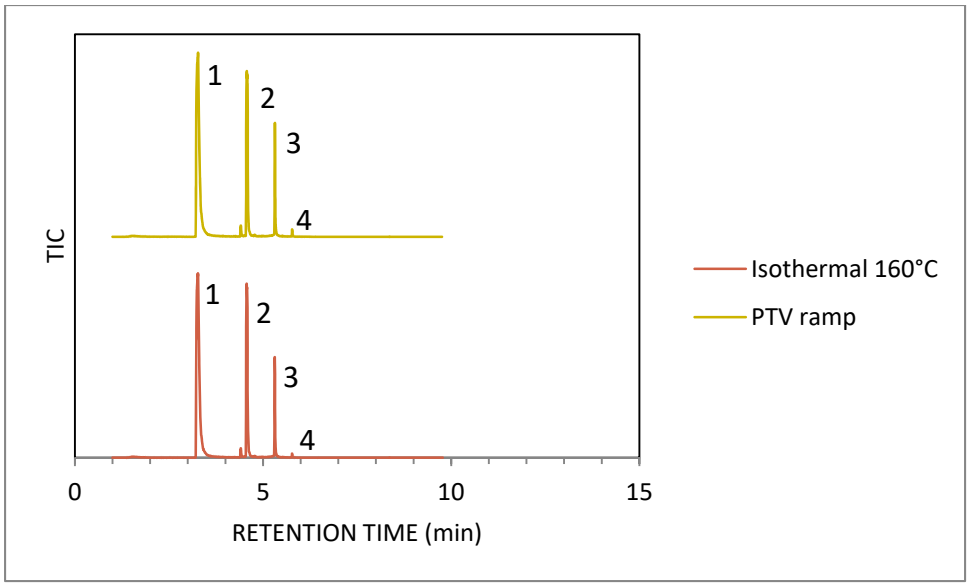


Figure 1-12: Comparison of Isothermal Inlet Temperature and a PTV Ramped Inlet for EGDN (1), NG (2), ETN (3), and PETN (4)

1.3.4 Sensitivity Comparison of Liquid Injection and TV-SPME

The increase in sensitivity that TV-SPME has over liquid injection has been documented over the past several years [15, 17].Previously it has been reported as being nearly 6 times as sensitive in the detection of nicotine and 12 times more sensitive for nitroglycerin[15, 17]. This method directly compares the sensitivity of EGDN and NG over the same linear range, as well as a look at the sensitivities at low concentrations. Over the range of 1ppm to 100ppb both liquid and TV-SPME produced a linear regression with high R^2 values. However, the slope of the lines as well as the signal to noise (S/N) at 100ppb suggest the dramatically lower limit of detection (LOD) attainable by TV-SPME compared to traditional liquid injection, this is showcased in Table 1-3.

Table 1-3: Comparison of calibration curves for liquid injection and TV-SPME of NG from 100ppb-1ppm

Injection Method	Slope	R^2	S/N at 100ppb
Liquid (splitless, 1µL)	4.2×10^3	0.975	119
TV-SPME (70µL)	1.4×10^4	0.999	1394

Lower concentrations were analyzed for both NG and EGDN for comparison outside of the calibration curve. The lowest comparable concentration for EGDN was 100ppt. Liquid injection had a S/N of 6 and TV-SPME had a S/N of 67. Similar results were seen for NG. At 500ppt liquid injection had a S/N of 0 (not detected) while TV-SPME still had a S/N of 35, well above the LOD S/N of 3. TV-SPME experiments were done at even lower concentrations. These resulted in detection of analyte as low as 50ppt for EGDN (S/N of 28) and 100ppt for NG (S/N of 9). These results fall well below the levels of explosive commonly recovered during analysis [21]. In general, the S/N in TV-SPME was eleven times higher than in traditional liquid injection. A comparison of the S/N generated by liquid injection and TV-SPME can be seen in Table 1-4.

Table 1-4: Low concentration signal to noise comparison of NG and EGDN for liquid injection and TV-SPME

Analyte	S/N For Liquid	S/N for TV-SPME
EGDN (100 ppt)	6	67
EGDN (50 ppt)	0	28
NG (500 ppt)	0	35
NG (100 ppt)	0	9

Figure 1-13 shows the peak that is seen at a concentration of 50 ppt for EGDN. The chromatogram has been zoomed in so that the peak can be clearly seen. The RT of this peak matches up with all previous runs of EGDN.

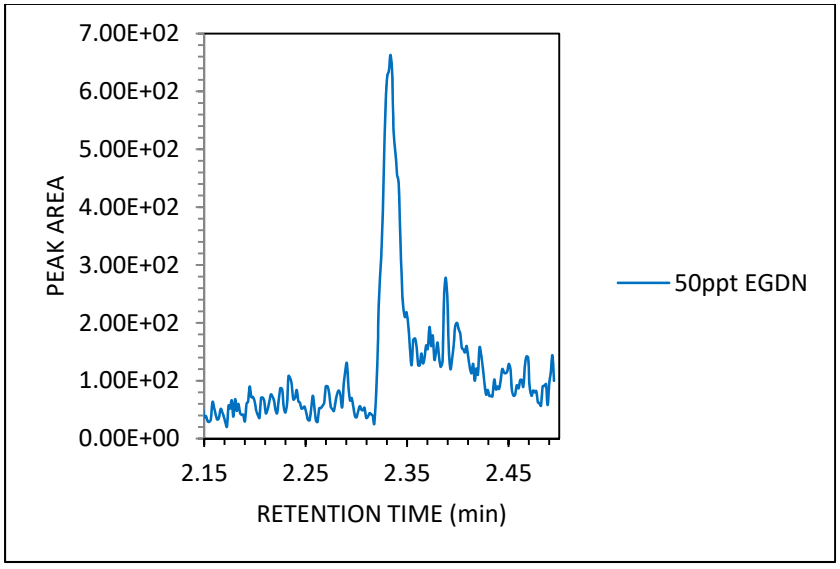


Figure 1-13: Instrument response for EGDN at 50 ppt

1.4 Conclusion

A TV-SPME GC/MS method was established and optimized for the trace detection of various nitrate esters. In particular, the impact of desorption temperature, desorption time, extraction temperature, and extraction time was studied. During a statistical optimization, it was determined that the desorption and extraction temperature parameters had the most impact on the signal as well as the degradation of the analyte. It was also shown how a PTV inlet can be used to cover a broad range of explosives while minimizing thermal degradation. TV-SPME was compared to liquid injection and was seen to have more than an 11 times greater S/N at low concentrations as well as detection of analyte as low at 50ppt.

This method could be adapted and used for “dirty” samples, such as soil samples recovered from the scene of an explosion. Adapting this method would cut down greatly on time spent cleaning up each sample for analysis prior to liquid injection as well as providing one method for the rapid detection of a broad range of explosives. Future steps for this project would be to analyze real world samples and to analyze a broader range of explosives to determine the overall versatility of this method.

CHAPTER 2. DESIGN AND IMPLEMENTATION OF DERIVATIZATION SCHEMES FOR COMMON ILLICIT DRUGS FOR ANALYSIS VIA GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

2.1 Introduction

Controlled substances make up a large portion of the work for a modern forensic laboratory. These drugs can vary greatly, from naturally occurring peyote, a small cactus that has psychoactive effects when ingested, to more sophisticated synthetic drugs like 25C-NBOMe, a synthesized drug made by substituting the amine in 4-chloro-2,5-dimethoxylphenethylamine (2C-C) with a 2-methoxybenzyl group[22]. Because of the variety of drugs that require analysis, a variety of techniques are in common use[5, 23]. Common screening techniques, also known as presumptive tests, include thin layer chromatography (TLC) or color tests. TLC utilizes a stationary phase typically made of a silica-based adsorbent in conjunction with a non-polar solvent as the mobile phase. This mobile phase travels up the plate via capillary action. The mobile phase will cause substances to travel up the plate at different rates based upon the competitive interactions with the mobile and stationary phase. This distance traveled can then be measured and compared to a standard.

Color tests involve placing a small amount of the suspected drug onto a spot plate and adding a drop of the reagent. Depending on the type of test and the drug that is present, a visible color will develop indicating a class of drug. The analyst can then decide on the next test based upon these results.

At least one confirmatory test must be done on all samples to identify a specific controlled substance. Two common confirmatory tests are Gas Chromatography Mass Spectrometry (GC/MS) and Fourier Transform Infrared Spectroscopy (FTIR)[5]. This project focused on GC/MS as the primary means of confirmatory testing.

The high temperatures that a GC/MS must reach for analysis can cause the decomposition of compounds, primarily in the inlet. This can be an issue during drug analysis as not every drug is thermally stable. Thus, the purpose of this project was to find a means to analyze common controlled substances and thermally labile controlled substance via GC/MS. Derivatization is a common way to take a substance that is not thermally stable and make it amenable to the high temperatures of a GC/MS[23]. Derivatization removes a labile hydrogen from a compound and replaces it with more a stable functional group.

The three primary mechanisms for this process are silylation, alkylation, and acylation. Silylation replaces a labile hydrogen with a silyl group (R_3Si-)[24]. Alkylation replaces a hydrogen with an alkyl group; the addition of a methyl group to carboxylic acids to form methyl esters is a common substitution with this reaction. Acylation substitutes the hydrogen with an acyl group, (R-(CO)-)[25]. Table 2-1 depicts the overall

reaction for each type of derivatization. The bold hydrogens represent the hydrogens involved in the reaction and "X" signifies either nitrogen or oxygen.

Table 2-1: Derivatization reactions for silylation, alkylation, and acylation

Derivatization Category	**Reaction**	**Products**
Silylation	R - X – **H** + $(CH_3)_3$-Si-Cl	R - X-Si-$(CH_3)_3$ + **H**Cl
Alkylation	R – (C=O) - O – **H** + CH_3OH	R – (C=O) - O – CH_3 + **H**OH
Acylation	R – X – **H** + CH_3(C=O)-Cl	R – X – (C=O)CH_3 + **H**Cl

There are numerous derivatization reagents on the market for each category of reaction[22, 24, 26-32]. However, this project used one reagent per category. For silylation N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was used. BSTFA is the derivatization reagent whereas TMCS acts as silylation catalyst for the process. Trifluoroacetic anhydride (TFAA) was used for acylation and alkylation used N, N-dimethylformamide dimethyl acetal (DMF-DMA). The reagents were chosen based upon their use in literature, the high volatility of their products, a rapid single-phase reaction, and versatility – ability to derivatize more than one type of functional group.

BSTFA is known to be very versatile with a variety of different types of compounds[24, 26, 30-31, 33-35]. The addition of TMCS expands this versatility even more. Products of BSTFA are generally more stable and more volatile than their underivatized forms. It is useful for both amines and hydroxyls. TFAA is commonly used with amines, such as methamphetamine and amphetamine[25, 36-37]. DMF-DMA is currently used to a

much lesser extent with controlled substances, but has proven effective in the derivatization of barbiturates, carbazepine, and artificial corticosteroids[38-41]. This reagent will be used as a novel way of derivatizing drugs of interest.

This project looked at three different categories of drugs totaling 15 different drugs of interest. The drugs chosen were based off common drugs analyzed as well as GC/MS "problem children", meaning drugs that are traditionally difficult to analyze via GC/MS or are not currently analyzed via GC/MS[42]. The drugs involved in this project are listed in Table 2-2.

Table 2-2: Summary of the drugs involved in the project with their structure and molecular weight sorted by compound class

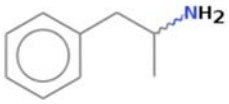
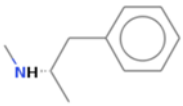
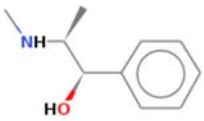
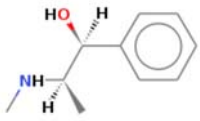
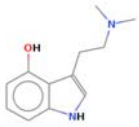
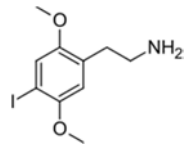
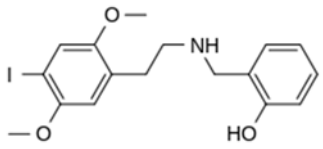
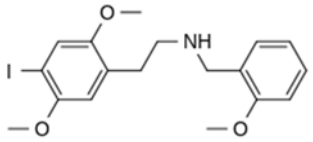
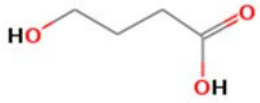
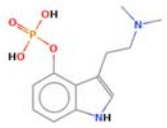
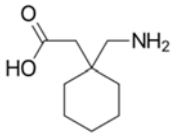
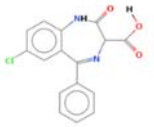
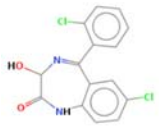
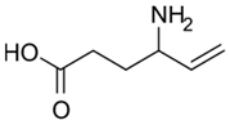
COMPOUND CLASS	DRUG	STRUCTURE	MW
Amines / Hydroxylamines	Amphetamine		135
	Methamphetamine		149
	Ephedrine		165
	Pseudoephedrine		165
	Psilocin		204

Table 2-2 Continued

Amines / Hydroxylamines	2C-I		307
	25I-NBOH		413
	25I-NBOMe		427
Carboxylic / Phosphonic Acids	GHB		104
	Psilocybin		284
Zwitterions (amine + carboxylic acid)	Pregabalin		159
	Gabapentin		171
	Clorazepate		314
	Lorazepam		320
	Vigabatrin		129

Several of these compounds are commonly analyzed via GC/MS, such as amphetamine and methamphetamine[43]. However, many are not due to thermal instability. Table 2-3 lists these drugs and the issue that causes them to be difficult to analyze via GC/MS.

Table 2-3: Examples of controlled substances for which GC/MS can be problematic

Compound(s)	MW (amu)	Issue
25I-NBOH	413	converts to 2,5 dimethoxy phenethylamine (2C-I)
Clorazepate	315	decarboxylates to N-desmethyldiazepam
Gabapentin	171	zwitterion that decomposes with the loss of water
Gamma Hydroxy Butyric Acid (GHB)	104	converts to Gamma Butyric Lactone (GBL)
Lorazepam	321	decomposes with the loss of water
Pregabalin	159	zwitterion that converts to 4-isobutyl-2-pyrrolidinone
Psilocybin	284	converts to psilocin
Vigabatrin	129	zwitterion that decomposes with the loss of water

Each mode of derivatization requires an agreeable functional group to allow for reaction, thus not all compounds will react with all three of the reagents chosen. Some will not work at room temperature and some required heating for the reaction to occur. TFFA with carboxylic acids is a good example of this. Though this reaction can occur, it can require your sample to be basified via liquid/liquid extraction prior to derivatization or require a high temperature and/or longer time[44]. Table 2-4 depicts all drugs and derivatization agents that were attempted in this project.

Table 2-4: Analytes and derivatization agents used

Analyte(s)	Functional Group(s)	Silylation	Alkylation	Acylation
amphetamine methamphetamine ephedrine pseudoephedrine psilocin	-OH -RN-H $-NH_2$	BSTFA + TMCS	N,N-dimethylformamide dimethyl acetal (DMF-DMA)	TFAA
25I-NBOH 25I-NBOMe 2C-I	-RN-H $-RNH_2$	BSTFA + TMCS	DMF-DMA	TFAA
Clorazepate	-RN-H -COOH	BSTFA + TMCS	DMF-DMA	TFAA
Gabapentin	$-NH_2$ -COOH	BSTFA + TMCS	DMF-DMA	TFAA
GHB	-COOH	BSTFA + TMCS	DMF-DMA	
Lorazepam	-RN-H -COOH	BSTFA + TMCS	DMF-DMA	TFAA
Pregabalin	$-NH_2$ -COOH	BSTFA + TMCS	DMF-DMA	TFAA
Psilocybin	$-O-H_2PO_3$	BSTFA + TMCS	DMF-DMA	
Vigabatrin	$-NH_2$ -COOH	BSTFA + TMCS	DMF-DMA	TFAA

The goal of this project was to complete phase one of a five phase project in analyzing difficult drugs via GC/MS. This phase was focused on finding an appropriate derivatization scheme for analysis on a GC/MS.

2.2 Materials and Methods

2.2.1 Materials

All drugs were in solid form upon receipt. Amphetamine hemisulfate salt, Methamphetamine HCl, Ephedrine HCl, Pregabalin, and Lorazepam were obtained from Sigma Aldrich. Pseudoephedrine HCl and Clorazepate dipotassium were obtained from Grace Chemical. Psilocin, 25I-NBOH HCl, 25I-NBOMe HCl, GHB sodium salt, Psilocybin, Gabapentin, Vigabatrin, and 2C-I HCL were obtained from Cayman Chemical. Liquid injection vials, caps, and insert were purchased from Phenomenex. BSTFA with 1% TMCS was purchased from Thermo Scientific in 1g ampules. Trifluoroacetic Anhydride (TFAA) and N,N-Dimethylformamide Dimethyl Acetal (DMF-DMA) were purchased in 1mL amounts from Sigma Aldrich. Acetonitrile (ACN) HPLC grade, Methylene Chloride (DCM) HPLC grade, and Methanol (MeOH) HPLC grade, were all purchased from Fisher Scientific. Chloroform was purchased from Sigma Aldrich.

2.2.2 Instrumental

An Agilent 6890N Gas Chromatogram (GC) coupled to an Agilent 5975 Inert Mass Selective Detector with a Gerstel MPS MutliPurpose Sampler was used for all analyses. All samples were injected via liquid syringe in either split mode (20:1 split ratio), or splitless mode (noted for each sample individually). The method used is based on the

standard method used by the Indiana State Police Forensic Science Laboratory with modifications. There were 2 methods that were used for all samples. Method 1 used an isothermal inlet temperature of 250°C and an oven program beginning at 90°C for 1 minute, ramp at 15°C/min to 280°C and hold for 1 minute. Method 2 used an isothermal inlet temperature of 270°C and an oven program that went to 300°C and held for 2 minutes. All other parameters remained the same between the two methods. The majority of all samples used Method 1, each sample is annotated with which method was used. Both methods also had the following GC parameters: column flow of 2.5 mL/min of hydrogen, mass transfer line at 280°C, and a solvent delay of 2 minutes. The MS was set to the following: source temperature of 230°C, full scan range of 20-550 in EI mode, normal scan speed, and EMV in the relative mode. The Autosampler used a 1 µL injection volume with 2 washes in Acetone before each sample, 1 wash with the sample itself, and 2 Acetone washes after each run. All samples were kept at room temperature during Autosampler procedures.

2.2.3 Sample Preparation

All raw samples were run in ACN, Methanol, or Chloroform, depending on their solubility. For derivatization reactions, 200 µL of each derivatization reagent was used. The amount of drug present for each reaction varied from 50 µg to 700 µg, dependent on the amount of drug available. The concentration of the solutions analyzed via GC/MS is denoted for each sample. Drugs that were dissolved in MeOH prior to derivatization

were placed in a blow-down apparatus until all solvent had been removed and only the drug was present for derivatization. Reactions with all reagents were done at 60°C for the amount of time suitable for each drug then 800 µL of ACN was added to bring the final volume of each sample to 1 mL.

2.3 Results and Discussion

2.3.1 Amines and Hydroxylamines

This category of compounds encompasses a large portion of the drugs that were investigated, including amphetamine, methamphetamine, ephedrine, pseudoephedrine, psilocin, 2C-1, 25I-NBOH, and 25I-NBOMe. Every drug was analyzed in the underivatized form as well as after reaction with each of the three reagents: BSTFA, TFAA, and DMF-DMA.

2.3.1.1 Amines and Hydroxylamines – Underivatized

Amphetamine, methamphetamine, ephedrine, pseudoephedrine, and psilocin were prepared in MeOH at 0.5 mg/mL and analyzed using Method 1 in split mode. An intense, single peak was produced for all of these samples. Each peak had a retention time that differed from the others, except for ephedrine and pseudoephedrine.

Ephedrine and pseudoephedrine are diastereomers and are not generally separated using GC[23]. Table 2-5 shows the chromatograms, mass spectra, and major ion fragmentations for each of these drugs that were identified successfully.

Amphetamine fragments due to alpha cleavage, forming a tropylium ion (m/z 91) and a fragment containing the side chain (m/z 44). In similar fashion, methamphetamine produces a tropylium (m/z 91) and a side chain fragment (m/z 58). Pseudoephedrine forms two primary fragment ions at m/z 77 and a m/z 58. This occurs via cleavage of the bond connecting the aromatic ring (m/z 77) to its side chain. There is also alpha-cleavage at the nitrogen producing the m/z 58 fragment. Ephedrine fragments in the same manner as pseudoephedrine; hence they cannot be differentiated from one another.

Psilocin produces two primary ions when fragmented, m/z 58 and m/z 204, with the latter being the molecular ion. The m/z 58 is formed by alpha cleavage at the nitrogen.

25I-NBOH was prepared as a 200 µg/mL solution and was analyzed using both methods in split and splitless mode, but produced only a small peak that was identified as 2,5 dimethoxy phenethylamine (2C-I), a known degradation product of 25I-NBOH[45]. 25I-NBOMe and 2C-I were prepared in the same manner as 25I-NBOH. 25I-NBOMe was analyzed using Method 2 in splitless mode and 2C-I was analyzed using Method 1 in splitless mode. Both compounds produced appropriate peaks.

25I-NBOMe is the first compound in this class that undergoes a more complicated fragmentation. Initial fragmentation occurs as expected as alpha cleavage

from the nitrogen producing the ion at m/z 150. This m/z 150 ion can also be formed upon the loss of iodine from the larger of the two fragments formed via the initial alpha cleavage. A loss of CNH_3 from this ion results in an ion at m/z 121[46]. 2C-I has a strong signal for the molecular ion in EI at m/z 307. Hydrogen rearrangement [46] allows for a loss of CH_3N resulting in the ion at m/z 278. A methyl group loss from this fragment provides the next fragment seen at m/z 263. An iodine ion can also be seen at m/z 127. The lower mass fragments at m/z 39, 51, 65, and 77 are representative of aromatics[46-47].

Table 2-5: Chromatograms, mass spectra, and fragmentation patterns for amines/hydroxylamines in the underivatized form

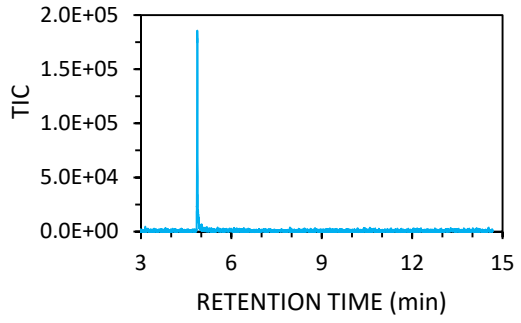
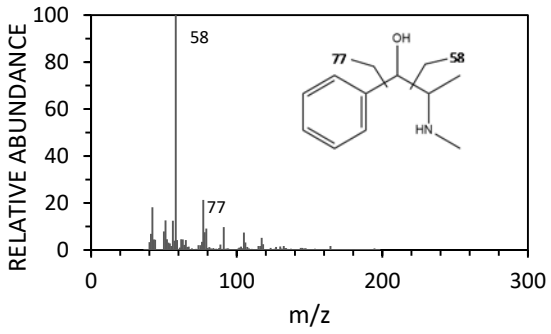
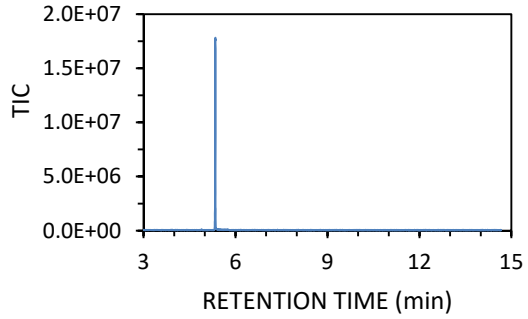
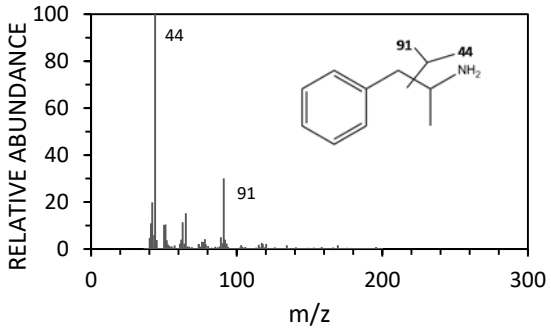
Chromatogram	Mass Spectrum and Fragmentation
Pseudoephedrine	
	

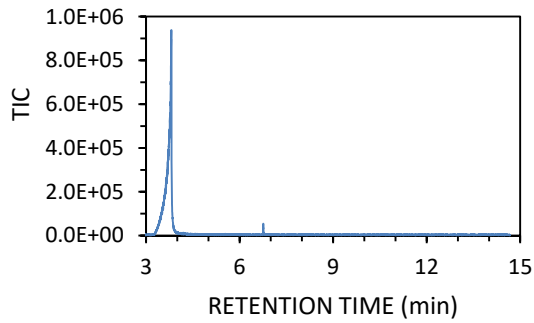
Table 2-5 Continued

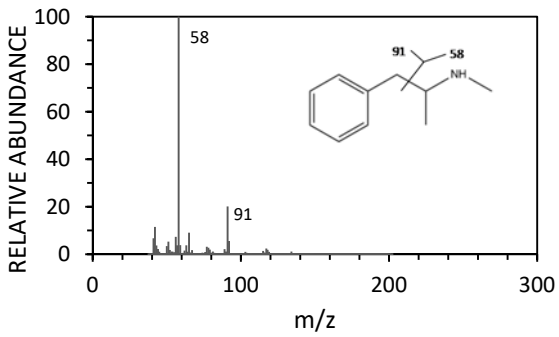
Amphetamine



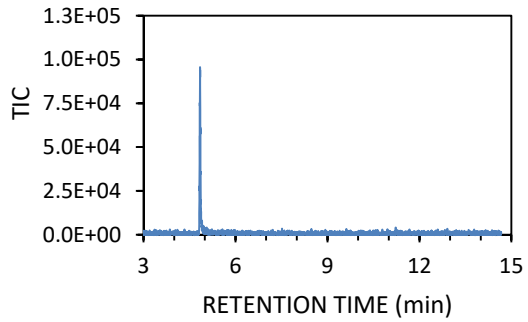


Methamphetamine





Ephedrine



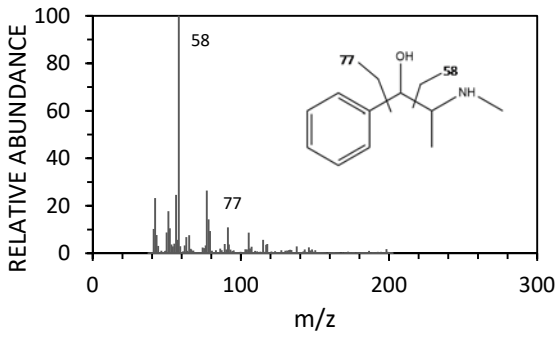
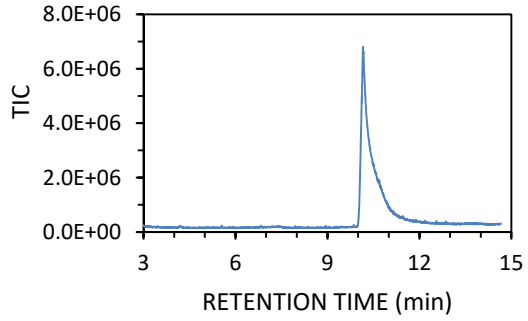
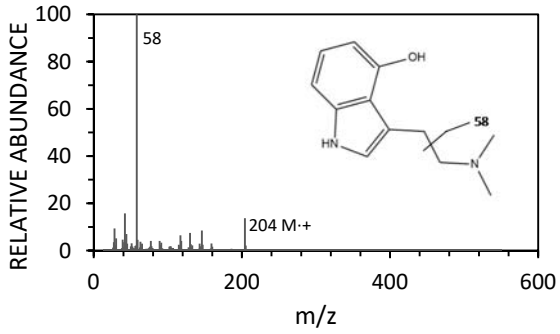


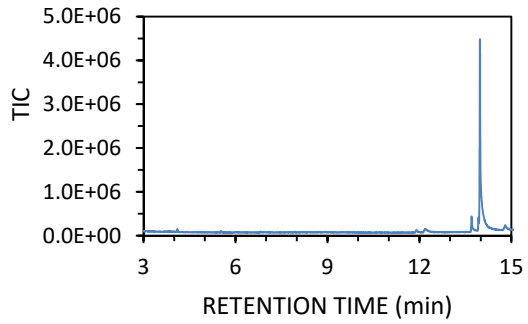
Table 2-5 Continued

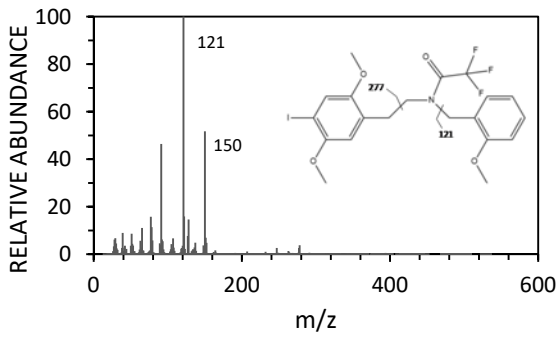
Psilocin



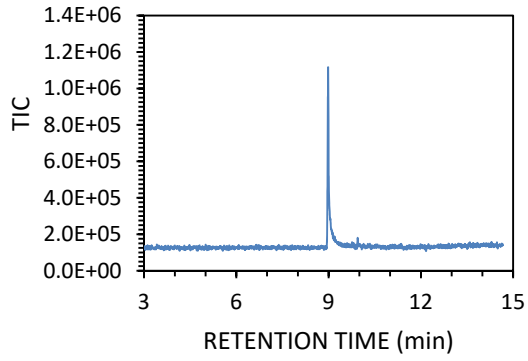


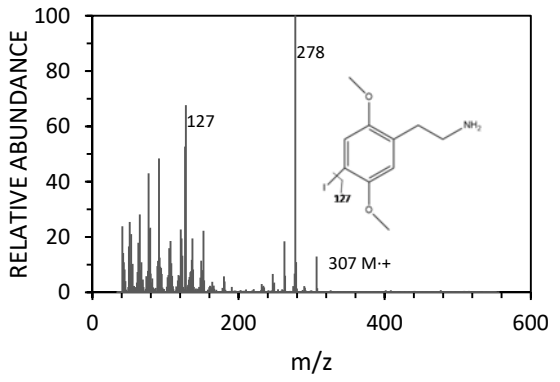
25I-NBOMe





2C-I





2.3.1.2 Amines/Hydoxylamines – Derivatized with TFAA

The most widely used derivatization reagent for this class of compounds is TFAA. The use of this reagent is well documented and TFAA was also found to be the best reagent for this group of compounds in this study. Every sample was derivatized directly by adding 200 µL of TFAA to the solid form of the drug. Each reaction was done at 60°C until the sample had fully dissolved, then 800 µL of ACN was added to reach the final concentration. If a sample did not dissolve after 1 hour, 800 µL of ACN was added to help facilitate the reaction per instructions provided with the reagents[44].

The same instrument methods and concentrations used for the analysis of the underivatized drugs were used during this analysis. The results are shown in Table 2-6. Due to TFFA derivatizing functional groups in s similar way each time, there are common ions that are seen as a result of products produced with this reagent. M/z 69 and 118 are two of the most common ions produced as a direct result of this.

In amphetamine, alpha cleavage results in m/z 91 (tropylium) and a m/z 140 base peak. Methamphetamine undergoes the same derivatization process where the $(CO)CF_3$ replaces the hydrogen on the nitrogen. Alpha cleavage then occurs resulting in the m/z 91 and m/z 154.

As discussed above, the underivatized forms of ephedrine and pseudoephedrine are diastereomers and co-elute. The two derivatized compounds produce the same mass spectra, but they have different RT's and are easily distinguished based upon this characteristic. One theory as to why this occurs is that once an additional 112 mass units

is added to the amine during the derivatization process, the difference in spatial orientation around the chiral center becomes great enough to affect the overall polarity of the molecule. This would cause each molecule to interact slightly differently with the stationary phase of the GC column and thus affect the RT's of each allowing for adequate separation[48]. Work has been done in the separation of enantiomers using a chiral stationary phase and derivatization via TFAA and Pentafluoropropionic Anhydride (PFPA)[49].

The TFAA derivatization of ephedrine and pseudoephedrine occurs at the amine nitrogen, replacing the hydrogen with a $(CO)CF_3$. The base peak of m/z 154 is a result of the loss of 17 from the hydroxyl and subsequent alpha cleavage at the nitrogen. This is seen in both ephedrine and pseudoephedrine.

Psilocin produced two chromatographic peaks during derivatization. Both peaks have similar mass spectra, however; the later eluting of the two shows a greater similarity to the raw drug than to the derivatized one. This is shown in the addition of higher mass fragments that are not seen in the derivatized product, but are seen in the underivatized form. However, the lack of the characteristic m/z 204 seen in the underivatized form and the addition of the m/z 69 may indicate partial derivatization. These observations together lend to the conclusion of a partially derivatized psilocin rather than an additional product being formed because of the derivatization process. A higher derivatization temperature, such as 80°C, could assist in encouraging this reaction to completion. The TFAA derivative of psilocin has the hydrogen from the hydroxyl replaced with $(CO)CF_3$. Upon ionization in the mass spectrometer, cleavage of

the perfluorinated carbon results in a m/z 69. The m/z 58 base peak is a result of alpha cleavage at the nitrogen.

The derivatization of 25I-NBOMe with TFAA gives a molecular ion at m/z 523. The reaction occurs at the nitrogen joining the two aromatic groups replacing the labile hydrogen with $(CO)CF_3$. Alpha cleavage at the nitrogen forms an ion at m/z 277. The side chain fragment from alpha cleavage cleaves at the nitrogen resulting in an ion at m/z 121, the base peak. This fragment consists of an aromatic ring with a methoxy and a methylene.

2C-I after derivatization with TFAA has a molecular ion at m/z 403. This then undergoes alpha cleavage at the nitrogen producing a fragment at m/z 277 and a fragment at m/z 126. The fragment at m/z 247 is a result of cleavage of two methyl groups from the m/z 277 fragment.

Table 2-6: Chromatograms, mass spectra, and fragmentation patterns for amines/hydroxylamines derivatized with TFAA

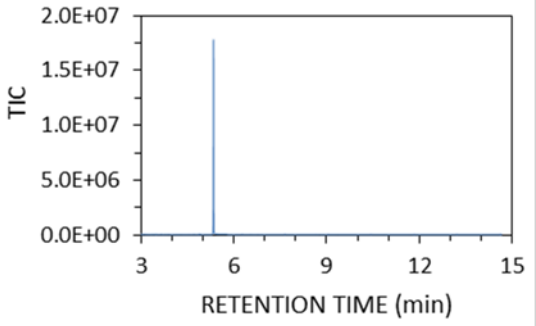
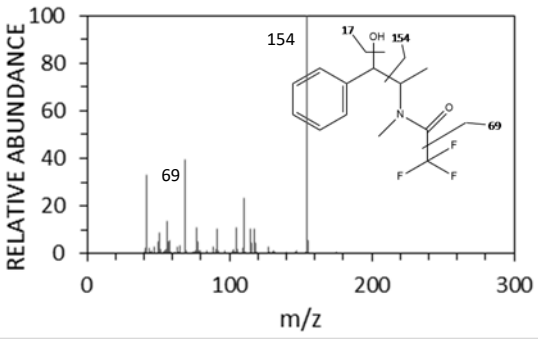
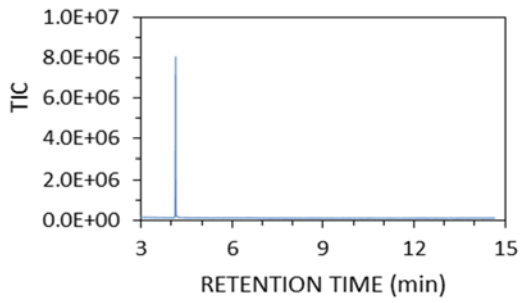
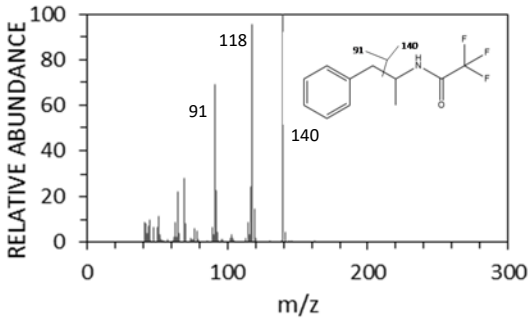
Chromatogram	Mass Spectrum and Fragmentation
Pseudoephedrine	
	

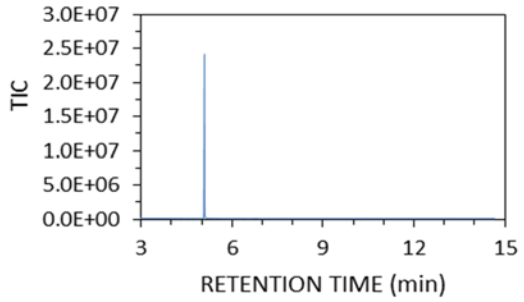
Table 2-6 Continued

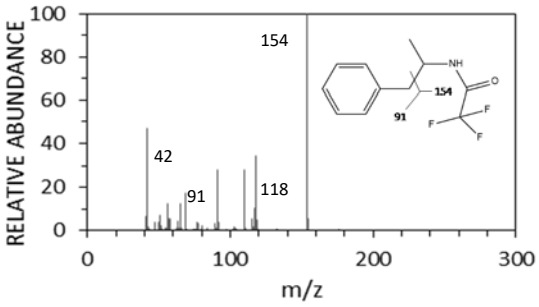
Amphetamine



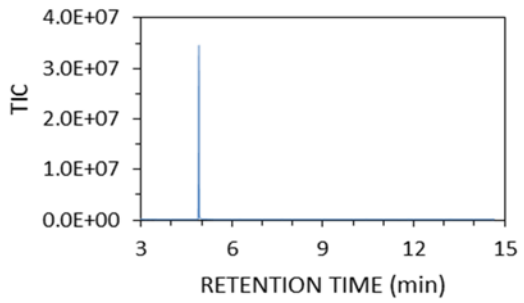


Methamphetamine





Ephedrine



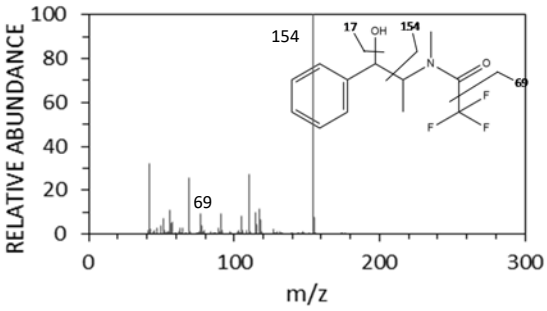
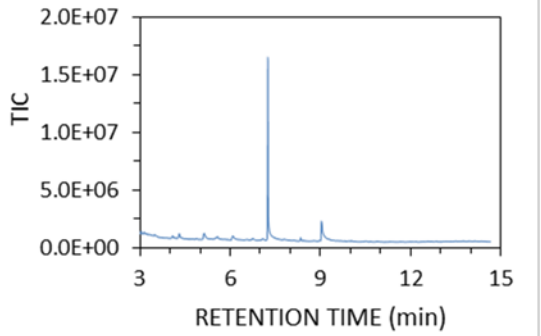
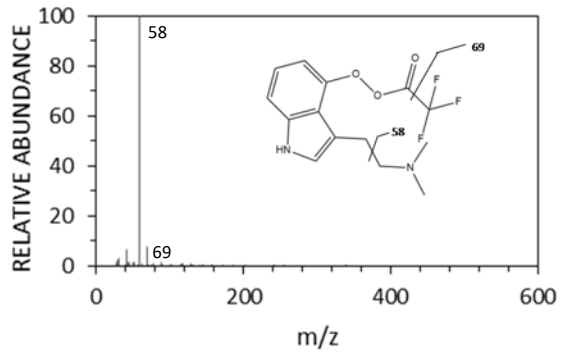


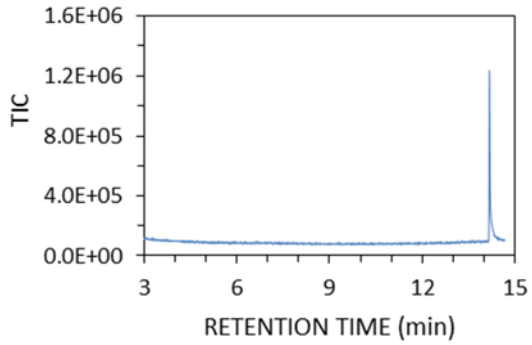
Table 2-6 Continued

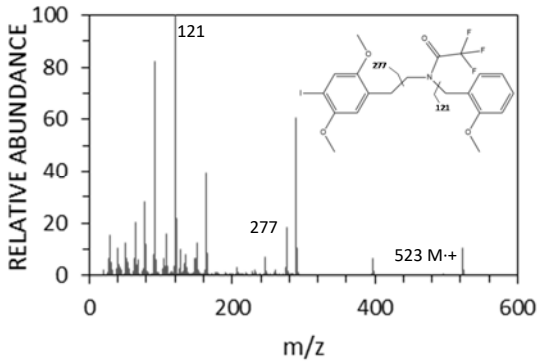
Psilocin



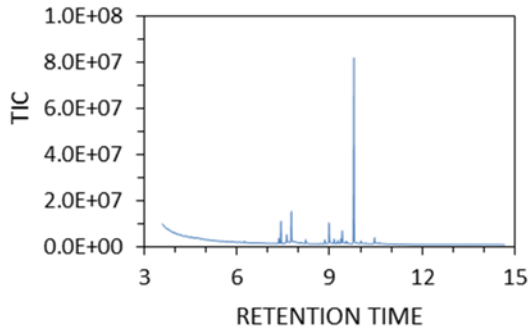


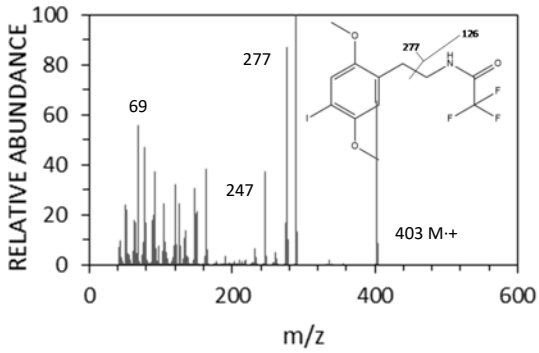
25I-NBOMe





2C-I



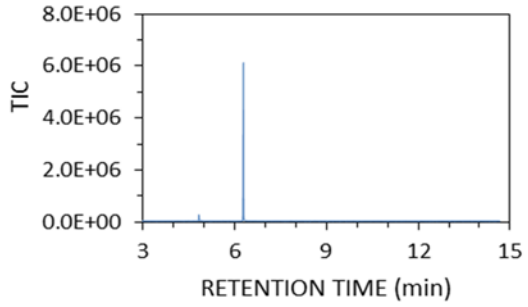
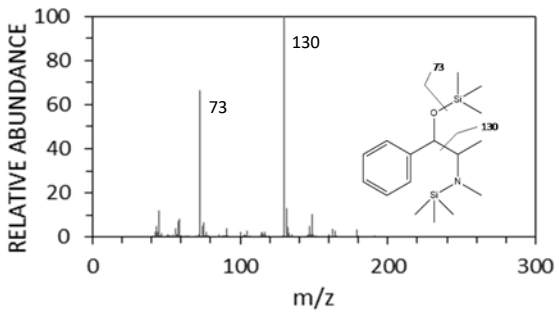
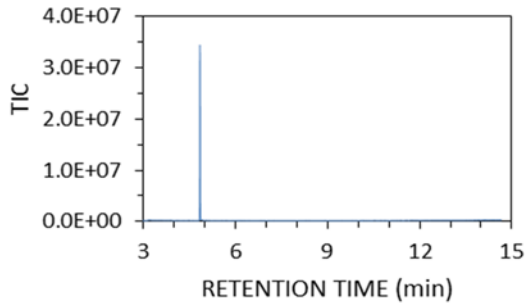
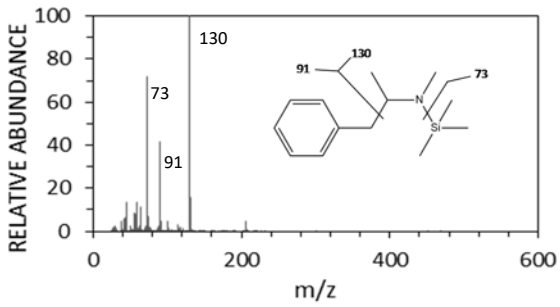


2.3.1.3 Amines/Hydoxylamines – Derivatized with BSTFA

BSTFA is a derivatization reagent that is known to work well for this class of compounds, though not to the same extent as TFAA. BSTFA worked well with pseudoephedrine and methamphetamine and less so with the other drugs in this class, as seen in Table 2-7. 25I-NBOH produced several peaks while using BSTFA, none of which could be identified as the derivatized compound. The reaction for amphetamine with BSTFA was not complete. The target compound was formed and identified, but the underivatized form of the drug was still present in the sample. Several attempts were made to force the reaction to completion (e.g., increase in reagent concentration, increase in temperature for the reaction, increase in the amount of time allocated for the reaction to reach completion), but none were successful.

All compounds that are derivatized with BSTFA have a labile hydrogen removed and replaced with a trimethlysilyl (TMS) group. This process produces an m/z 73 upon fragmentation common to TMS derivatives. Pseudoephedrine gains two TMS groups when reacted with BSTFA, one for the amine nitrogen and the other for the hydroxyl. The cleavage of one of these groups' results in an ion at m/z 73 and alpha cleavage at the nitrogen producing the base peak of m/z 130. Methamphetamine undergoes the same process where the hydrogen on the amine gets replaced with a TMS group. The same cleavage is seen here as it was in pseudoephedrine. The addition of the fragment at m/z 91 from the tropylium is the main difference.

Table 2-7: Chromatograms, mass spectra, and fragmentation patterns for amines/hydroxylamine's derivatized with BSTFA

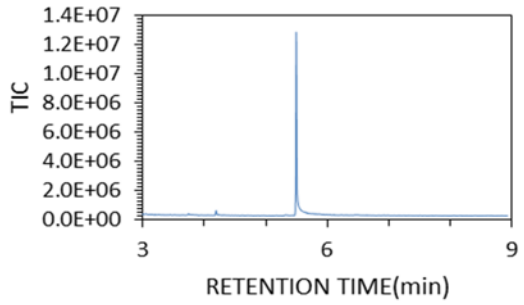
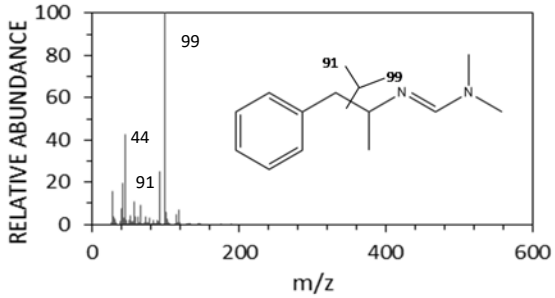
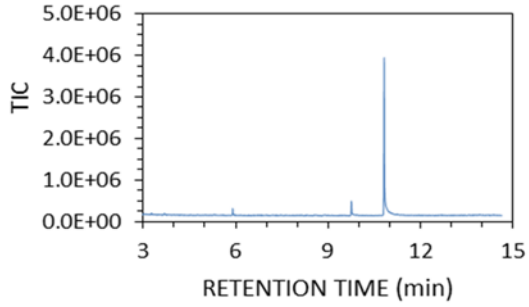
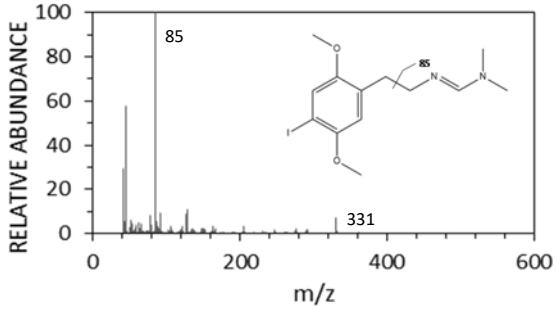
Chromatograms	Mass Spectra and Fragmentation
Pseudoephedrine	
	
Methamphetamine	
	

2.3.1.4 Amines/Hydoxylamines – Derivatized with DMF-DMA

DMF-DMA is not widely used and therefore represents a novel way of derivatizing amphetamine and 2C-I, as illustrated in Table 2-8. This process can produce ions during fragmentation common to many compounds, similar to BSTFA and TFAA, the most prevalent of these being m/z 44. Both compounds produced a main peak with several minor peaks; however the derivatized product could be identified by the mass

spectra. 25I-NBOH produced several peaks while using DMF-DMA, none of which could be identified as the derivatized compound.

Table 2-8: Chromatograms, mass spectra, and fragmentation patterns for amines/hydroxylamines derivatized with DMF-DMA

Chromatograms	Mass Spectra and Fragmentation
Amphetamine	
	
2C-I	
	

The identification of the reaction products of DMF-DMA with amphetamine and 2C-I both rely heavily on appropriate mass spectra interpretation, as these products are not currently known. These compounds follow the same derivatization reaction seen in amino acids[50]. This means that the primary amine will lose two hydrogens, which are replaced with a dimethylaminomethylene (DMAM) group. A carbon-nitrogen double bond is thus formed, making the derivative an imine[48]. In amphetamine there is no

molecular ion seen, but alpha cleavage at the nitrogen originally present in amphetamine results in a fragment at m/z 91 (tropylium) and m/z 99, the base peak. The peak at m/z 44 is alpha cleavage from that same nitrogen but cleaving after the carbon in the DMAM.

2C-1 undergoes the same reaction that amphetamine does. The same alpha cleavage paths results in the base peak of m/z 85 and the characteristic m/z 44. There is no molecular ion seen in 2C-I, however there is an ion at m/z 331. This fragment is a result of the molecular ion losing 31 mass units in the form of methoxy loss from the aromatic ring.

2.3.2 Carboxylic/Phosphonic Acids

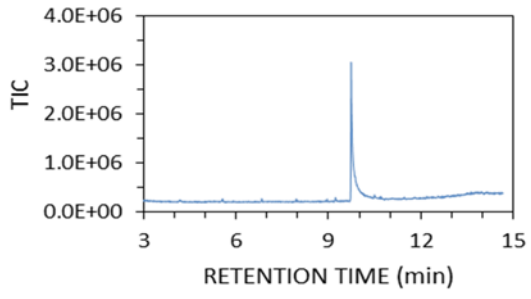
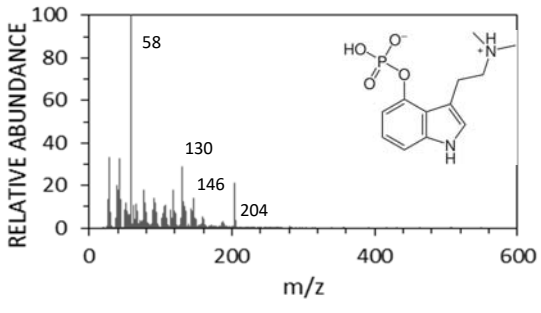
2.3.2.1 Carboxylic/Phosphonic Acids – Underivatized

Carboxylic and phosphonic acids make up the smallest category of compounds studied during this study - GHB and psilocybin. GHB and psilocybin were each prepared as a 0.5mg/mL solution in MeOH and analyzed using Method 1 with a splitless inlet. As has been previously reported [31, 33], GHB converts to gamma-butyrolactone (GBL) in the GC inlet. Only psilocybin could be identified in the underivatized form.

A surprising result was that the RT for psilocybin shown in Table 2-5 is 9.73 min whereas the RT for psilocin in Table 2-9 is 10.15 min. Differentiating these two

compounds in the underivatized form by GC/MS is generally considered to be impossible. The mass spectra for psilocin and psilocybin can also be differentiated, although this depends upon examining the relative intensities of the ions that are present in both mass spectra. In psilocybin, the phosphate group is lost first as a neutral loss[46], leaving a hydroxyl group. This generates the same structure as psilocin, thus the difficulty in differentiating the two. However, there are differences in the intensities of ions as a result of psilocybin having a positive charge on the amine. This causes far more fragmentation and thus higher intensities of each of the fragments as they are more abundant than what is seen with psilocin. Psilocin displays very low intensities for most fragments outside of m/z 58, the base peak formed, and m/z 204, the molecular ion. Psilocybin shows similar ion formation, but with a much more intense 204, 146, and 130. Lastly, the differences between psilocybin and psilocin also reflected in the spectra from the NIST MS database.

Table 2-9: Chromatograms, mass spectra, and fragmentation patterns for carboxylic/phosphonic acids in the underivatized Form

Chromatograms	Mass Spectra and Fragmentation
Psilocybin	
	

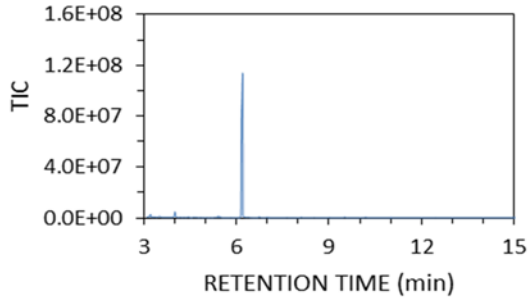
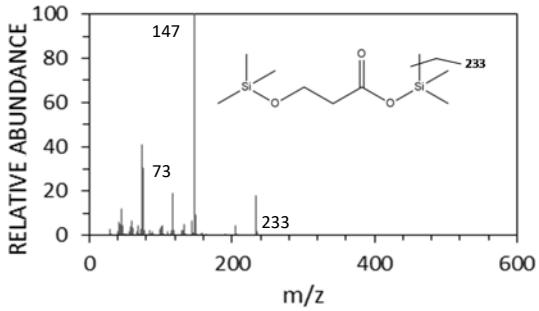
2.3.2.2 Carboxylic/Phosphonic Acids – Derivatized (All Reagents)

GHB is commonly derivatized by BSTFA to form GHB-TMS_2[51]. This can be seen in Table 2-10. GHB was not successfully derivatized by DMF-DMA. GHB was derivatized directly by 200 µL of BSTFA until dissolution was observed. 800 µL of ACN was added to bring the concentration of the solution to approximately 0.5 mg/mL. GHB-TMS_2 seems to undergo degradation when held at higher temperatures; this was seen during initial analysis of the compound. Due the short retention time of this compound, the initial oven temperature was lowered to 50°C for all experiments involving any form of GHB, in order to allow the compound to elute at a lower temperature. All other parameters remained the same.

The structure of GHB-TMS_2 is well understood, but the fragmentation of this derivative is generally not. Both the carboxylic acid and hydroxyl groups lose their hydrogen to a TMS group in this reaction. The first ion at m/z 233 is a result of cleavage of a methyl group on one of the TMS groups. The base peak of m/z 147 is a resultant of rearrangement causing a loss of $C_5H_{13}Si$ (m/z 101)[46-47]. The m/z 73 is the TMS fragment common to all compound derivatized with BSTFA.

No successful derivatization of psilocybin was accomplished using the 3 reagents in this study. Reaction with DMF-DMA produced virtually no signal and reaction with BSTFA produced several peaks, none of which could be contributed to the target compound. However, the successful derivatization of psilocin using TFAA allows for the differentiation between the two compounds.

Table 2-10: Chromatograms, mass spectra, and fragmentation patterns for carboxylic/phosphonic acids derivatized with BSTFA

Chromatograms	Mass Spectra and Fragmentation
GHB	
	

2.3.3 Zwitterions

2.3.3.1 Zwitterions – Underivatized

Zwitterions were the last class of compounds that was examined. The compounds in this class have both a positive and a negative charge resulting in an overall neutral molecule. The majority of these compounds contain both an amine and a hydroxyl group and both BSTFA and DMF-DMA were effective at converting these compounds into a derivative that was amenable to GC/MS.

The only compounds to produce an appropriate peak in the underivatized form were gabapentin and lorazepam. This was not expected as both are said to decompose with the loss of water in the inlet. However, both were clearly identified with appropriate match scores in the NIST database. Each compound exhibits a loss of water

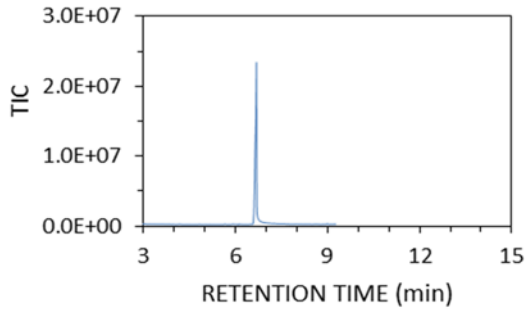
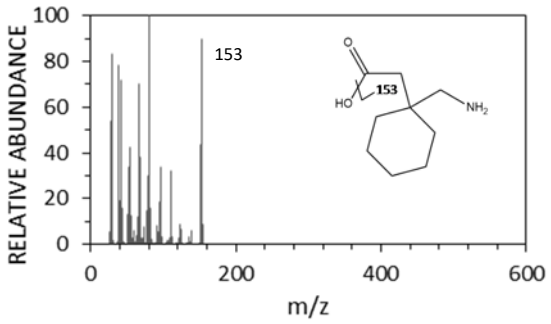
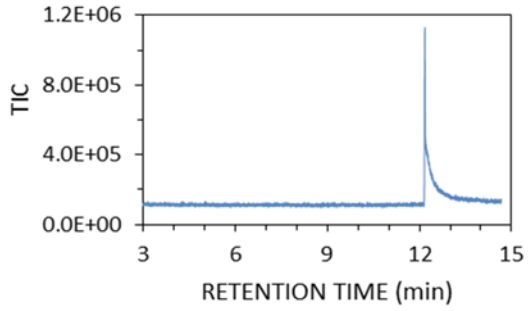
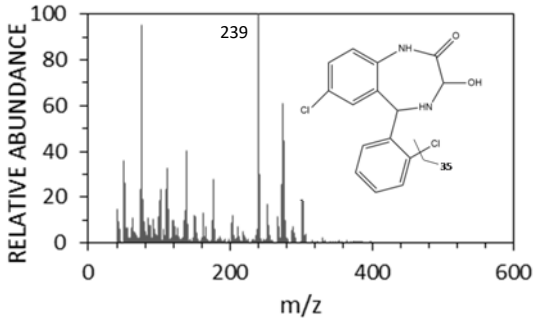
in the mass spectrometer, forming an ion at (M-18). Purchasing standards of the dehydrated forms of these drugs would allow for determination of the point of decomposition. If the RT's for the dehydrated standards line up with the RT's already seen then it could be concluded that dehydration is occurring in the inlet. If they do not line up then dehydration would be happening at the point of ionization.

Specifically, gabapentin undergoes a loss of water resulting in an ion at m/z 154, the remaining structure cyclizes via hydrogen rearrangement[46] prior to the cleavage of the cycloalkane. The low mass ions seen in the mass spectrum are characteristic of the fragmentation of cycloalkanes[46].

Lorazepam has a low mass ion series that is indicative of aromatic compounds (m/z 50, 51, 63, 64, 74, 75, and 76)[46]. This most likely arises from fragmentation of the aromatic ring following the alpha cleavage from the nitrogen. Based on the isotopic ratio of chlorine the number of chlorines present in each ion can be determined[46]. ^{37}Cl is approximately 1/3 the abundance of ^{35}Cl, therefore an ion at two mass units higher than the target ion that is roughly 1/3 the abundance of the target ion would contain 1 Cl. If this fragment is 2/3 the abundance then it would contain 2 Cl. Thus it can be seen than the first Cl is lost when forming the m/z 239 ion. Therefore, the base peak involves a loss of 1 Cl and an additional 48 coming from cleavage at the 2 nitrogen's.

Vigabatrin, pregabalin, and clorazepate did not produce any results in the underivatized form. All drugs were prepared as 0.5 mg/mL solutions in MeOH using Method 1 with a splitless inlet; the results are shown in Table 2-11 for gabapentin and lorazepam.

Table 2-11: Chromatograms, mass spectra, and fragmentation patterns for zwitterions in the underivatized form

Chromatograms	Mass Spectra and Fragmentation
Gabapentin	
	
Lorazepam	
	

2.3.3.2 Zwitterions – Derivatized With BSTFA

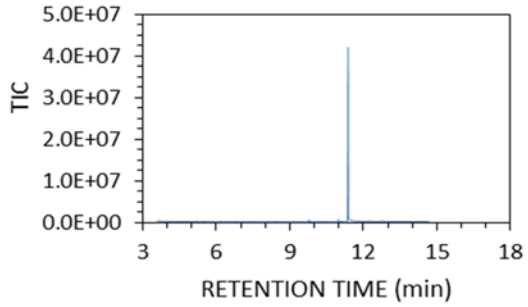
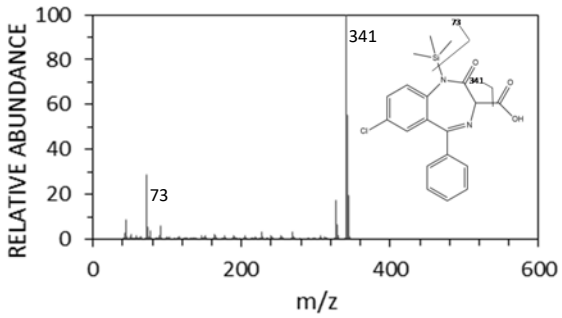
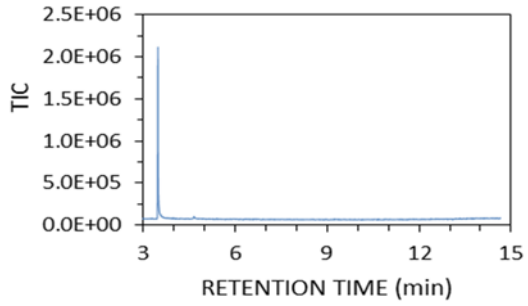
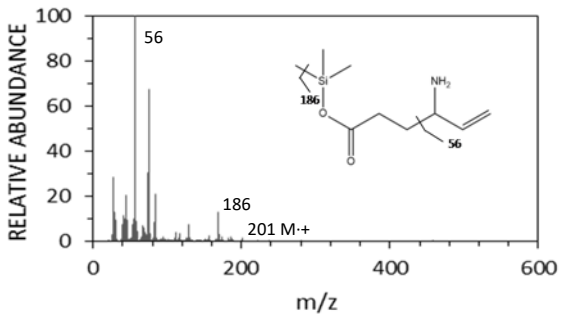
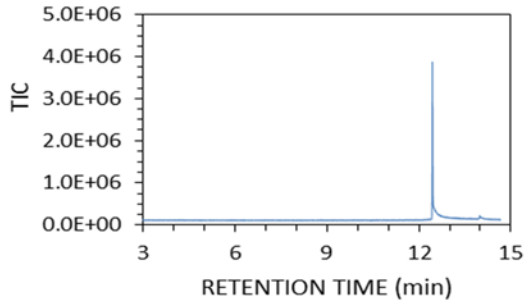
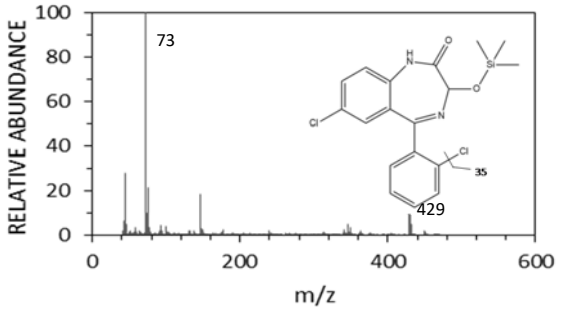
Using BSTFA for derivatization with zwitterions worked well with clorazepate, lorazepam, and vigabatrin. Each drug was derivatized directly with 200 µL of BSTFA and held at 60°C until dissolution was completed. Once completed 800 µL of ACN was added to bring the concentration of each analyte up to approximately .5 mg/mL. The results of the successful reactions are shown in Table 2-12.

Pregabalin produced several peaks because of derivatization with BSTFA. The target compound was able to be identified, but due to the presence of small amounts of the underivatized drug and many additional peaks, it was not considered a successful reaction. This drug shows the ability to be derivatized via this method, but ultimately may require a different reaction scheme than what was used in this project.

In clorazepate the TMS group preferentially silylates the amine. The ion at m/z 341 shows the same pattern previously discussed with the chlorines in lorazepam, indicating m/z 341 contains one chlorine. Cleavage occurring at the carboxylic acid results in the m/z 341 ion. The m/z 73 is produced via the cleavage of the TMS group.

Vigabatrin shows a rare molecular ion for this reagent at m/z 201, this is a result of silylation of the carboxylic acid. A loss of a methyl group produces the m/z 186 ion. From the m/z 186 a loss of 17 is observed in the form of ammonia following hydrogen rearrangement[46]. The base peak of m/z 56 is formed by alpha cleavage at the nitrogen. The first ion in Lorazepam, at m/z 429, is the result of a loss of one of the chlorines. This follows the same rules for isotopic ratios as previously seen with an isotopic ion two mass units higher and at one third the abundance of the ion of interest.

Table 2-12: Chromatograms, mass spectra, and fragmentation patterns for zwitterions derivatized with BSTFA

Chromatograms	Mass Spectra and Fragmentation
Clorazepate	
	
Vigabatrin	
	
Lorazepam	
	

2.3.3.3 Zwitterions – Derivatized With DMF-DFA

Derivatizing zwitterions with DMF-DMA, as with all the other compound classes, is a new method for derivatizing drugs. Gabapentin and vigabatrin were both successfully derivatized with this reagent. The same scheme was used for this reaction that was used with the previous reactions. The results can be seen in Table 2-13.

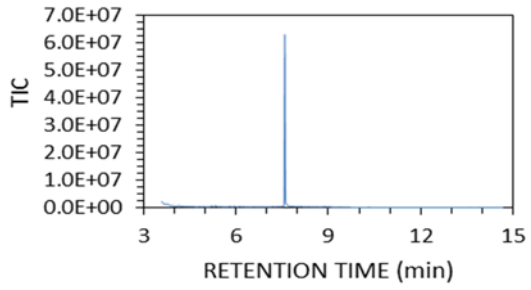
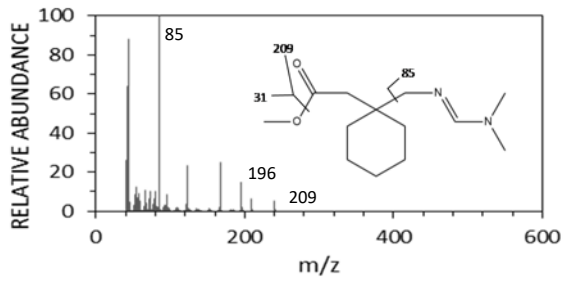
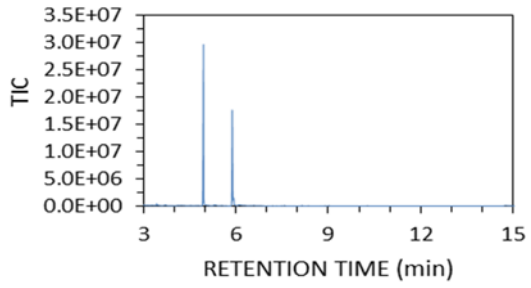
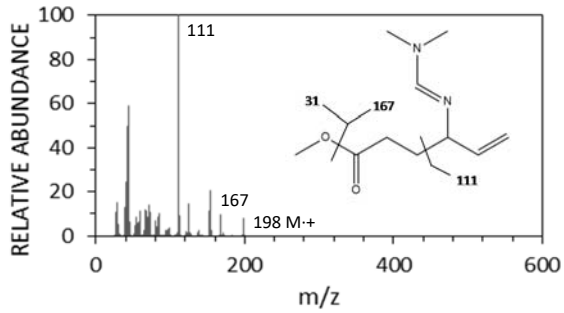
Gabapentin and vigabatrin contain both a primary amine and a carboxylic acid, making these most analogous to the reaction of DMF-DMA with amino acids [50]. The amine undergoes the same process discussed with the hydroxylamines and the carboxylic acid is methylated with the replacement of the hydroxyl group with a methoxy.

Gabapentin produces a singular clean peak, making identification a simple process in this case. The first primary fragment that is seen is m/z 209 which is the result of the molecular ion, 240 mass units, losing 31 mass units in the form of a methoxy. The molecular ion can be seen in a very small abundance. The base peak of m/z 85 is a result of alpha cleavage at the original nitrogen. This cleavage also leaves a m/z 196 that is also seen in small abundance.

Vigabatrin produces several peaks during this process, but all of the peaks can be accounted for as bi products commonly observed with this reaction. Vigabatrin undergoes the same derivatization process that gabapentin does. The molecular ion of m/z 198 is seen. The ion at m/z 167 is a result of the loss of a newly formed methoxy, as

seen previously. The base peak of m/z 111 is formed via alpha cleavage at the nitrogen originally present.

Table 2-13: Chromatograms, mass spectra, and fragmentation patterns for zwitterions derivatized with DMF-DMA

Chromatograms	Mass Spectra and Fragmentation
Gabapentin	
	
Vigabatrin	
	

2.4 Conclusion

The goal of this phase was to determine a derivatization technique that would work for each of the drugs listed and if one scheme could theoretically work for an entire class of drugs. Overall, amines/hydroxylamine's worked well with the TFAA reagent, as was expected. BSTFA was also seen to work well. A new derivatization reaction was found for amphetamine and 2C-I via DMF-DFA. All the compounds

analyzed in this class, apart from 25I-NBOH, were successfully derivatized using at least one of the reagents. Due to the successful use of TMS derivatives in this class, N-Methyl-N-tert-butyldimethylsilyltrifluoroacetamide (MTBSTFA) could be looked at as a viable alternative to BSTFA. MTBSTFA forms products more slowly, but the dimethyl tert-butylsilane derivatives are known to be much more stable than their TMS analogs. GHB was the only carboxylic/phosphonic acid that formed a successful derivatized product. BSTFA was used to form the well documented GHB-TMS_2. Psilocybin did not form a derivatized product, however. The key issue with this drug is the ability to detect it and differentiate it from psilocin. This was accomplished in two ways. The first of these being the ability to differentiate between the two in the raw form based on retention time and differences in the relative abundances of the fragment ions. If the analyst cannot reach a conclusion based on these results, the compound can be further verified by derivatizing it with TFAA. Only psilocin can be derivatized with this reagent. Both of these methods successfully demonstrate the differentiation that was sought between these two compounds.

Zwitterions were able to be derivatized by a greater variety of reagents. A novel way of derivatizing some of these compounds was found with DMF-DFA. In some cases, these reactions formed several peaks, but all were able to be accounted for as common bi-products seen when using this reagent. BSTFA also worked well with the majority of the compounds in this class, with the exceptions of gabapentin and pregabalin. The appropriate derivative was formed when attempting to derivatize pregabalin with

BSTFA, however the reaction was incomplete. This is another scenario where utilizing MTBSTFA could prove beneficial in forming a complete derivative.

DMF-DFA was found to be a novel way of derivatizing some drugs to much success. Any compound that contains a primary amine in this study undergoes the same process with this reagent. Both of the hydrogens from the primary amine are lost and one of the hydrogens from the alpha carbon in the DMAM group are lost, forming a nitrogen-carbon double bond. In compounds that contained both a primary amine and a carboxylic acid the carboxylic acid underwent a replacement of the hydroxyl group with a methoxy group. The initial loss of 31 mass units from the methoxy group was common followed by traditional alpha cleavage at the original nitrogen from the primary amine.

Table 2-14 presents a summary of all compounds that were successfully analyzed, both in the raw form and derivatized. In each case, the primary fragments that are formed in EI are also listed.

Table 2-14: Table of all compounds successfully analyzed and their primary fragments formed sorted by compound class

Compound	Derivatization Reagent	Primary Fragments Formed (m/z)
Amphetamine	Raw	**44**, 91
	BSTFA	**116**, 73, 91
	TFAA	**140**, 118, 91
	DMF-DFA	**99**, 44, 91
Methamphetamine	Raw	**58**, 91
	BSTFA	**130**, 73, 91
	TFAA	**154**, 42, 118

Table 2-14 Continued

Ephedrine	Raw	**58**, 77
	BSTFA	**58**, 73, 91
	TFAA	**154**, 110, 69
Pseudoephedrine	Raw	**58**, 77
	BSTFA	**130**, 73
	TFAA	**154,** 110, 69
Psilocin	Raw	**58**, 204
	TFAA	**58**, 69, 42
2C-I	Raw	**278**, 128, 91
	BSTFA	**174**, 73, 86
	DMF-DFA	**85**, 44, 331
	TFAA	**290**, 277, 403
25I-NBOH	Raw	Not Detected
25I-NBOMe	Raw	**121**, 150, 91
	TFAA	**121**, 91, 290
GHB	Raw	Not Detected
	BSTFA	**147**, 73, 117
Psilocybin	Raw	**58**, 130, 204
Pregabalin	Raw	Not Detected
	BSTFA	**174**, 73, 147
Gabapentin	Raw	**81**, 153, 30
	DMF-DFA	**85**, 44, 167
	TFAA	**178**, 81, 67
Clorazepate	Raw	Not Detected
	BSTFA	**341**, 73, 327
Lorazepam	Raw	**239**, 75, 274
	BSTFA	**73**, 429, 147
Vigabatrin	Raw	Not Detected
	BSTFA	**56**, 75, 73
	DMF-DFA	**111**, 44, 42

**Grey boxes indicate multiple peaks present or incomplete derivatization*

***All ions are listed in decreasing order of abundance, with the base peak being listed first and in bold*

CHAPTER 3. FUTURE DIRECTIONS

3.1 Explosives Project

During this project a method was developed for the separation and identification of 7 nitrate ester explosives. This method is yet to be proven with real world samples and this is the next logical step to be taken. Samples have been collected with the help of the Indiana State Police from devices utilizing Semtex 10 (PETN), Semtex 1H (PETN and RDX), and Detasheet (PETN). Soil samples as well as pieces of the devices were recovered from the scene and are being stored for analysis. Instrument difficulties did not allow for the examination of these samples during the duration of this project. EPA Method 8095, Explosives by Gas Chromatography, outlines traditional GC procedures for identification of explosive residue. This coupled with EPA Methods 3500 (Organic Extraction and Sample Preparation), 3600 (Cleanup), 5000 (Sample Preparation of Volatile Organic Compounds) and 8000 (Determinative Chromatographic Separations) would provide a solid foundation for designing a procedure for the analysis of soil and debris samples. These methods outline extraction procedures for a variety of types of samples. Within this falls explosive residue found in soul[19]. The procedure involves a

solvent extraction, filtration, and concentration prior to experimentation. By using TV-SPME the clean-up steps of these methods could be eliminated. The nature of TV-SPME and its inherent increase in sensitivity over traditional liquid injection could also potentially provide the sensitivity necessary to analyze samples directly without the need for pre-concentration.

This method shows extreme sensitivity for both NG and EGDN using Chemical Ionization (CI). Determining the true LOD of these analytes and all other nitrate esters would not only be beneficial but necessary for this method to be used in a forensic science laboratory. The detectable levels for NG and EGDN, as seen in this project, fall well below the average level of NG that is recovered from pipe bombs [21]. Coupled with this, the LOD of each analyte in EI mode should be investigated to determine the viability of this method in EI. This is due to the fact that EI is the preferred ionization source used by forensic science laboratory. Prior to attempting to determine the true LOD of the method an additional optimization should be done. All four analytes can be analyzed as standards, establishing their retention times (RT) as well as the RT's of their degradation products, prior to optimization. This would allow for parameters that would theoretically be closer to ideal for all four analytes.

The application of this method to analytes outside of the seven examined here would be necessary prior to adoption by forensic science laboratories. Additional nitramines, such as HMX, RDX, and Tetryl should be analyzed via this method to determine their viability with it, as well as explosives such as trinitrotoluene (TNT), hexamethylene triperoxide diamine (HMTD), and triacetone triperoxide (TATP). HMTD

and TATP are thermally labile and are traditionally analyzed using LC/MS [4]. However, this method has been seen to analyze other thermally sensitive explosives like pentaerythritol tetranitrate (PETN) and erythritol tetranitrate (ETN) to much success.

The original goal of this project was to take a separation technique and apply it to samples that are collected using the M-Vac system. The M-Vac system is a device that was designed for the extraction of DNA from porous material. The system applies a buffer to the material and suctions it off into a container. This buffer then can be utilized by a laboratory technician to extract the DNA that could be potentially present. This system would be of great use to a forensic scientist in scenarios like the Boston bombing where a device was placed in a backpack prior to deflagration. The pieces of the backpack could be gathered and extracted by this system. During this extraction, it is hoped that DNA would not be the only thing recovered, theoretically residue from the explosive filler used would be recovered in this buffer. After the extraction of DNA from the buffer this could then be transferred to an explosive analyst for potential identification of explosive residue.

An additional area that could be explored would be utilizing a Vacuum Ultraviolet (V-UV) detector in place of or in addition to the mass spectrometer. The UV spectrum obtained by traditional UV-Vis detectors is limited by the amount of interference present in air. This then results in a low end wavelength of around 250 nm. Nitrate esters do not show a great deal of unique absorbance at this level. Applying a vacuum to the system allows for the detector to utilize shorter wavelengths in the UV spectrum, around 120 nm. Nitrate esters produce a much more active absorbance spectrum at

these lower wavelengths. This includes transitions between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of σ and σ* bonds of the carbon backbone. Preliminary work with a V-UV detector from VUV Analytics shows that it is possible to distinguish between nitrate esters using this type of detector. This is seen in Figure 3-1. Nitrate esters will fragment into a m/z 46 and a m/z 72 during EI fragmentation and into m/z 46 and m/z 62 during CI fragmentation. Regardless, these compounds cannot be differentiated via mass spectral analysis alone. This problem is not seen with a VUV detector. This is due to the change in absorbance that is seen based on spatial orientation of functional groups and the number of functional groups present. These together allow for an analyst to distinguish between identically fragmenting nitrate esters using a VUV detector as opposed to a mass spectrometer.

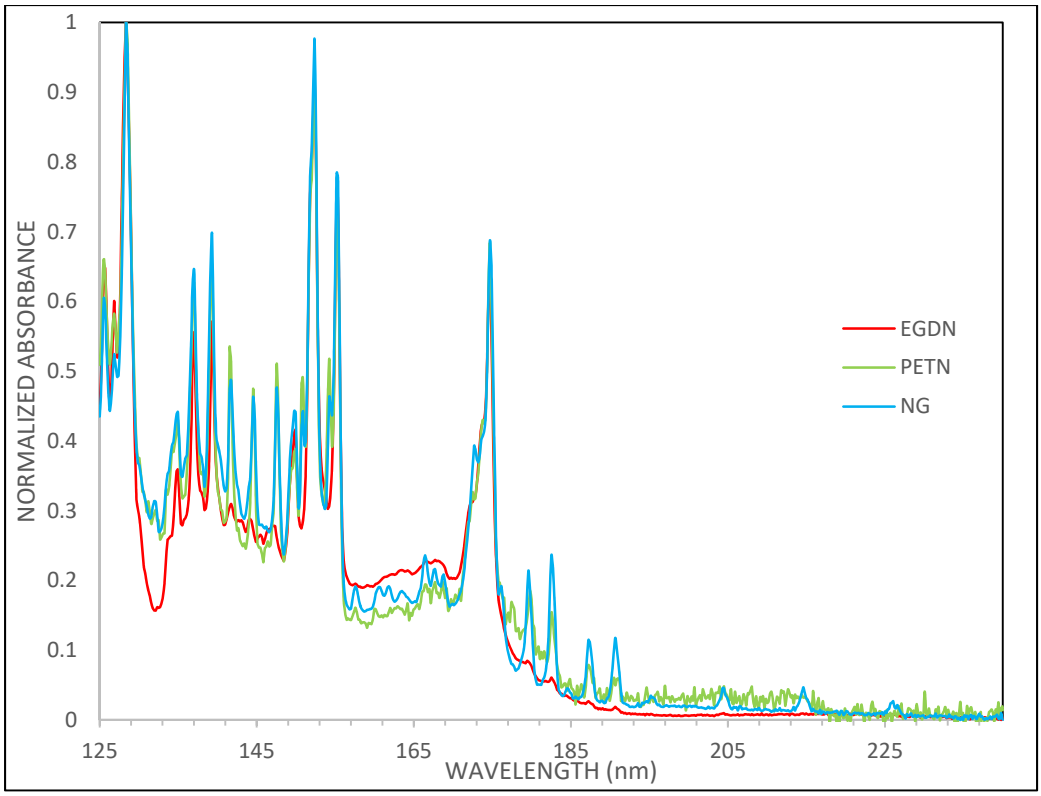


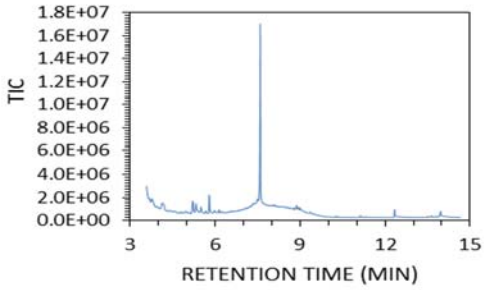
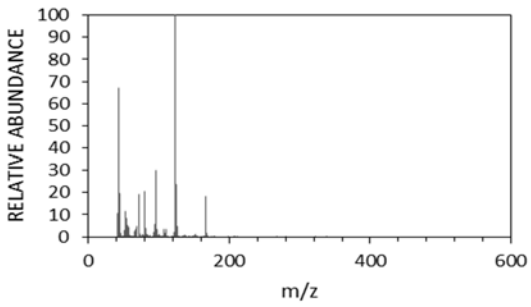
Figure 3-1: V-UV spectra of EGDN PETN and NG

3.2 Controlled Substances Project

This project devised various derivatization schemes for many different illicit drugs, the most novel of which was the use of DMF-DMA. Success was seen with primary amines and zwitterions (primary amines with a carboxylic acid). Moderate success was also seen with other types of compounds, specifically with secondary amines and hydroxyl's. The chromatograms for these substances had many peaks produced and not all of them were able to be identified. This suggests that with more work the procedure could be altered to facilitate the full reaction of DMF-DMA with these drugs. Using a

different solvent or a higher reaction temperature could help facilitate this. If a higher temperature is attempted, 100°C is recommended as this temperature has been documented as helping to facilitate the complete reaction of amino acids that prove difficult to derivatize with this reagent[52]. The addition of pyridine is also commonly seen with derivatization reaction to help encourage the process. Table 3-1 depicts the results of clorazepate.

Table 3-1: Chromatogram and mass spectrum of assumed DMF-DMA derivative of clorazepate

Chromatograms	Mass Spectra and Fragmentation
Clorazepate	
	

TV-SPME, that was used in the analysis of explosives, and has been used for other forensically relevant samples, could be used for the analysis of illicit drugs. TV-SPME has been proven to yield a significant increase in instrument response when compared to liquid injection. It also has the added benefit of being able to introduce "dirty" or unfiltered samples into a GC/MS. This would be able to help eliminate work up required by analysts and reduce solvent waste due to the small amount of volume needed for TV-SPME. Using an automatic sampler coupled to a GC/MS using SPME would also help limit the amount of analyst time that is needed to perform analysis on

each sample. This is due to the ability of SPME to do on-line derivatization[33, 53]. The SPME fiber can be exposed to the derivatization agent prior to being exposed to the analyte, thus causing the derivatization to occur on the fiber. This could also have the added benefit of decreasing the amount of time needed for derivatization. By placing both the analyte and the reagent in the gaseous phase, and thus in a higher energy state, the reaction should proceed much quicker than what was seen during bench-top wet chemistry reactions.

Preliminary work has also been in the direct derivatization of phenethylamines with derivatization agent. Specifically, 50 mg of amphetamine hemi-sulfate was added to a headspace vial. Separately 200 µL of TFAA derivatization agent was added to a different vial and placed in the incubator at 60°C. A Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) fiber was exposed to the reagent for 0.1 minutes. The vial containing the amphetamine hemi-sulfate was then incubated for 60 seconds at 60°C prior to the SPME fiber being exposed to it for 20 minutes. The appropriate peak for Amphetamine-TFA was observed in extreme abundance. Continued experimentation with additional compounds that worked adequately with TFAA as well as incorporating common adulterants and cutting agents into analysis is highly encouraged.

3.3 Hydrogen Sulfide Project

Necrotizing Enterocolitis (NEC) is a disease that causes bacteria to enter into the intestines and can cause inflammation and infection. This can then spread throughout the bowels and intestines. It is an issue that affects approximately 16% of all very low birth weight infants that are born at Riley Children's Hospital[54]. Currently the primary mode of correction is an intestinal resection at the site of the diseases. This can cause issues later in life for the child. Thus, a way to correct this disease without the removal of part of the intestines would be highly advantages.

A working theory of Dr. Troy Markel at the Indiana University School of Medicine is that by encouraging the paracrine release of hydrogen sulfide a decrease in proapoptotic signaling and a decrease in proinflamatory cytokine production would be seen. This would then lead to an improved recovery without surgical removal of the diseased tissue. To do this, a quantitative way of determining minute changes in hydrogen sulfide concentrations would need to be established.

There are many established ways to quantitate hydrogen sulfide, the majority of which fall into the photometric class[55-60]. The Methylene blue test is the most common photometric approach used[55]. This test involves aqueous hydrogen sulfide reacting with N, N-dimethylphenyl-1, 4-diamine in the presence of ferric ions to cause a quantifiable blue color to be produced. The blue color is a result of the formation of heterocyclic thiazine dye that is formed as a result of this reaction. This can then be measured through a variety of techniques, such as: UV-Vis spectroscopy, fluorescence, or HPLC.

This methodology has been seen to have a limit of detection of 0.01 parts per million (ppm) using traditional UV-Vis Spectroscopy.

Hydrogen sulfide is volatile, but breaks down rather quickly in solutions. Also, due to its extreme volatility, there is sample loss as long as it is in open air. Both of these lend to the idea that hydrogen sulfide should be converted into something more stable. By converting this into something more stable it also makes it more amenable to analytical techniques such as GC/MS.

Preliminary work has been with quantifying this compound using GC/MS. Pentafluorobenzyl Bromide (PFBBr) was chosen as an alkylation reagent for hydrogen sulfide due to its previous applications to sulfur containing compounds. Converting H_2S into a much larger thermally stable molecule via PFBBr allowed for analysis by GC/MS. Samples were obtained from the Markel group for analysis and quantitation. The samples were in Minimum Essential Medium Alpha (1X) from gibco and contained cell lines that were either stimulated or not stimulated with several different kinds of drugs thought to increase the production of hydrogen sulfide. 900 µL of sample was frozen immediately after being allowed to grow overnight. The frozen samples were what were received. These samples were then processed through a derivatization reaction prior to analysis. Since the samples were provided as an aqueous solution, the analyte needed to partition into an organic layer for derivatization via PFBBr. Thus 1 mL of a basic solution containing a Phase Transfer Catalyst (PTC) was added to the 0.9 mL of sample. Making the solution basic causes the hydrogen sulfide to become sulfide ions that can then be “carried” to the organic layer via the PTC. Several PTC’s were tested:

benzalkonium chloride, hexadecyltrimethylamonium bromide, and tetrabutylammoniumhydrogen sulfate. Benzalkonium chloride was selected due to it being the only PTC that did not cause precipitation when used in conjunction with the growth media. To this solution 2 mL of a 2% v/v solution of PFBBr in toluene with decafluorobiphenyl as an internal standard was added to form two distinct layers. This internal standard was used based on its non-interaction with PFBBr and its solubility in the sample matrix. Toluene, dichloromethane, and acetone were all tried as potential solvents. Acetone did not form two distinct layers, dichloromethane is denser than the media, and thus toluene was used for ease of extraction. This two layer system was then vortexed for 15 seconds prior to being placed on a shaker table for 1 hour. After one hour an aliquot of the organic layer was added to a GC vial for analysis via liquid injection GC/MS.

The derivatization of hydrogen sulfide with PFBBr produced a compound containing one sulfur and two pentafluorobenzyl rings. This compound is depicted in Figure 3-2.

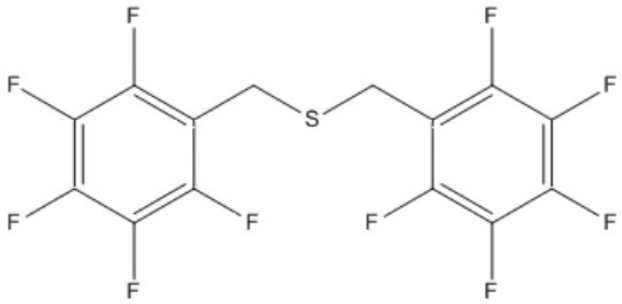


Figure 3-2: Depiction of the compound produced via PFBBr alkylation of hydrogen sulfide

Once retentions times were determined for this compound and the internal standard all future analysis was run in Selected Ion Monitoring (SIM). The SIM parameters were set to m/z 334 for the internal standard and m/z 181 and m/z 394 for the analyte. Initial work was promising due to the ease of detection of this analyte. The concentrations present in the sample fell well above the limit of detection for this method.

The potential issues that were experienced were with extraction efficiency, sample degradation, and background levels of hydrogen sulfide. Background levels of hydrogen sulfide are a confirmed issue that was faced. The media that was used to grow the cells has an inherent background signal of hydrogen sulfide of around 0.1ppm. However, the level of background analyte was not consistent from sample to sample. This could be a result of extraction efficiency, sample degradation, or inconsistencies in sample preparation. Because of this, accurate quantitation was not possible as there

was no way to subtract the background signal from the signal purposefully produced. Attempts were made to account for variations in background signal by running multiple media blanks, but the error bars produced for these samples overlapped with the concentration determined to be in the stimulated samples. Preliminary data with various media and means of inducing sulfide production were collected, however the data was inconclusive.

The next step for this project is recommended to be Total Vaporization Solid Phase Microextraction. This method of sample introduction was previously discussed in Chapter 1. As discussed in section 3-2, TV-SPME can be adapted to use on-line derivatization. This could potentially be of great benefit to this project. The two main issues seen with the initial work were with extraction efficiency and sample degradation. Extraction efficiency could essentially be eliminated as an issue by informing the lab techs at the time of sample prep to freeze a smaller amount of sample, as decided upon by the equation defining TV-SPME sample volume, and heating that sample directly until total vaporization. This would cut down immensely on the time that the sample has to degrade prior to derivatization as well as eliminate the matrix effects that could be affecting extraction efficiency. The sample could also be spiked with an internal standard prior to thawing. The SPME fiber would then be exposed to the headspace of a vial containing PFBBr that has been heated. The fiber, now having PFBBr adsorbed onto it, would then be exposed to the vial containing the vaporized analyte and internal standard. Derivatization with PFBBr is a fast process and this should be sufficient to derivatize the analyte for analysis. This would also increase the sensitivity of the method.

By increasing the sensitivity the slope of a calibration curve would increase dramatically, thus potentially allowing for the differentiation of concentrations that reside close together on a calibration curve, such as 0.10 ppm and 0.15 ppm.

Using TV-SPME introduces several parameters not previously investigated as part of method development. Through initial experimentation with on fiber derivatization of illicit drugs, the following parameters are recommended: incubation temperature of 110°C, pre-fiber derivatization time of 0.1 minutes, polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber type, adsorption time of 20 minutes, desorption time of 60 seconds. All incubated samples should be agitated at the rate of 10 seconds on and 1 second off for the duration of incubation. Initial incubation of the sample should 60 seconds. This will allow sufficient time for thawing and vaporization to occur. The formula for volume of sample for TV-SPME at 110°C using water calls for 16 µL of sample. To this, 10 µL of the internal standard would be added at a concentration of 20 ppm in acetone.

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VITA

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PUBLICATION

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1. Sauzier, G.; Bors, D.; Ash, J.; Goodpaster, J. V.; Lewis, S. W., Optimisation of recovery protocols for double-base smokeless powder residues analysed by total vaporisation (TV) SPME/GC-MS. *Talanta* **2016,** *158*, 368-374.