DETERMINATION OF BIOGENIC AMINES IN DECOMPOSITION ODOR USING GAS CHROMATOGRAPHY MASS SPECTROMETRY

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Science in Chemistry.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation/Symbol	Definition
HRD	human remain detection
VOCs	volatile organic compounds
GC-MS	gas chromatography-mass spectroscopy
FAA	tetrafluoroacetylacetone
PFPA	pentafluoropropionic anhydride
SPME	solid phase microextraction
ST	sorbent tube
FID	flame ionization detector
IR	infrared spectroscopy
AS	amine standard
MS	mass spectroscopy
IBC	isobutyl chloroformate
IS	internal standard
Sy	the standard deviation of y-intercepts of regression lines or the standard error for the y estimate
S	the slope of the line
LOD	limit of detection
LOQ	limit of quantification
k	the number of measurements made to the unknown sample
n	the number of data points in the calibration curve
m	the slope of the line
y	the average y of the unknown
$\bar{y}$	the average y from the calibration curve
x_i	the individual points on the calibration curve
$\bar{x}$	the mean value of x from the points on the calibration curve

ABSTRACT

DETERMINATION OF BIOGENIC AMINES IN DECOMPOSITION ODOR USING GAS CHROMATOGRAPHY MASS SPECTROSCOPY

Mackenzie Wisor, Master in Chemistry

Western Carolina University (July 2021)

Director: Dr. Nuwan Perera

In a forensic investigation of missing persons or a suspected murder the detection of the corpse is often the most important piece of evidence.[1] Human remain detection dogs (HRD) are the most common method used in locating human remains.[2] Although these dogs are useful in distinguishing between animal and human remains little is known about how they are able to do this. The HRD dog's ability to detect human remains depends on volatile organic compounds (VOCs) that are emitted during decomposition.[3] Previous studies reported that a variety of different compounds including acids, alcohols, ketones, sulfides, and nitrogen containing compounds are present in decomposition odor.[3,4] Although these researchers found a variety of compounds they do not report the presence of biogenic amines like putrescine and cadaverine. These amines are produced during the decomposition process through decarboxylation of amino acids and is responsible for the distinct odor released by decomposing corpses.[5] Our hypothesis is that these biogenic amines are not being detected due to the limitation of the analytical methods used by other researchers. Primary amines often have high basicity, high polarity, and low

volatility compared to other VOCs, they tend to absorb and decompose in the GC column, sample vessels, and injection system making them hard to detect in low concentrations and difficult to analyze using GC-MS.[6] To obviate this problem, our goal is to develop an analytical method to analyze these amines by derivatizing them before the introduction into the GC system. Reacting with a suitable derivatization agent these amines can be converted into more volatile and less polar derivatives making analysis by GC-MS a feasible method.[6] Several derivatization methods were used. One was a method that used tetrafluoroacetylacetone (FAA) in a Schiff base reaction and another used pentafluoropropionic anhydride (PFPA) in an acylation reaction and neither produced the results that was desired so a different method was used.[6,7] The current reaction is formation of carbamates using chloroformates.[6] This reaction is particularly used on primary, secondary, tertiary amines, and is generally less reactive with water.[6] To use a chloroformate derivatizing agent it has to be in a pH controlled environment to complete the reaction.[6] To extract them for analysis via liquid-liquid extraction toluene is used.[8] Isobutyl chloroformate was the specific derivatizing agent used and was chosen for its higher peak response with the derivatives.[8] This has yielded clear and constant data that has indicated that the amines are being derivatized by the isobutyl chloroformate and that they can be detected in the GC-MS reliably. A calibration curve was made to fulfill the second goal of derivatization which is to quantify the amines. This method was also used on various soil samples and pseudo scent products to explore the practical application of this derivatization method. Amines were found in most of the practical samples tested and were quantified.

CHAPTER 1: INTRODUCTION

Motivation

Forensic investigation of a missing person often requires finding of the human remains. The use of human remain detection (HRD) dogs or cadaver dogs is one of the most commonly used methods that investigators use to find decomposing human bodies.[2] The department of justice considers the evidence that is provided by human remains are important when investigating a crime.[1] Some studies shows that cadaver dogs can recover human remains buried in a significant depth.[9] Although cadaver dogs show a remarkable ability to detect decomposing human bodies, the scientific basis for how they perform that task is largely lacking. Knowing how these HDR dogs detect human bodies by analyzing the composition of what is released during composition we can better train these dogs more safely and efficiently.

GC-MS is the method of choice for the analysis of compounds present in decomposition odor and several sampling techniques including solid phase microextraction (SPME) and sorbent tube (ST) has been used in previous research reported in the literature.[10–14] It is the ideal instrument because of the advantages it provides such as high resolving power, high sensitivity and most importantly its short analysis time and low cost.[6] Although previous researchers found a variety of compounds in decomposition odor including carboxylic acids, aldehydes, ketones, sulfides, and alcohols, none of these studies have reported the presence of amines that are responsible for the distinct odor of decomposing bodies. These known amines including cadaverine, putrescine, tyramine, histamine, spermine and spermidine are produced in decomposing bodies as a result of the degradation of proteins.[2] Identification and quantification

all amines that are released during decomposition could facilitate the development of more accurate HDR training aids. Previous studies show that there is a significant difference between the chemical composition of these artificial scents and the real decomposition odor.[11,15] The reason that GC-MS hasn't been used for detecting amines is that they are slightly polar and are more soluble in water which damages the GC-MS. The amines also have a tendency to stick and decompose onto the column and not vaporize properly which results in poor data.[6] These problems are easily sidestepped if the amine is derivatized into a more volatile and nonpolar form before analysis.[6] This makes analysis of amines by GC-MS not only possible but the best option in the quest of identifying and quantifying unidentified amines.

Access to a corpse in a criminal investigation is often required for a missing person or homicide investigation.[2] To protect fragile evidence that may be present on the body non-invasive methods are preferred when locating buried remains.[2] Some common methods are ground searches, photographic techniques, geophysical methods, remote sensing and HRD dogs. HRD dogs are particularly useful due to their ability to search a large area, detect even skeletonized remains, and locate remains that were buried at significant depths.[2,9,16] In order to train these dogs to locate human remains a variety of methods are used. Using resources at a body farm (like the one at WCU) is an ideal situation but due to various legal and ethical issues surrounding human remains this isn't always possible. Because of this issue trainers will try to obtain biological samples such as blood, bones or tissues for training. Others prefer commercial odor mimics (pseudo corpse) to protect themselves and their dogs from the hazards of using biological material.[15]

In one study conducted, HRD dogs that were trained with real human remains were sent to find the artificial scent training aids but were unable to locate them.[11,15] This means that the

artificial scents are missing key components that are crucial to the scent of a decomposing corpse. In a study conducted on the analysis of cadaver dog training aids, researchers were not able to detect these amines using a headspace two dimensional (2D) GC-MS method in two types of pseudo corpses.[15] With previous analysis of decomposing corpses researcher's results often didn't include putrescine and cadaverine even though they are known to be released during the decomposition process, and this is due to their method of analysis.[5] This drives home the fact that not only are the pseudo corpses a simplified imitation of real human corpses but that there is much research left to do before an accurate and standard pseudo corpse is ready.[15]

Amines are formed by decarboxylation of amino acids during decomposition and are responsible for the smell.[5] If putrescine and cadaverine, the structures of which are provided in Figure 1, were not detected during the analysis, which means that other biogenic amines may have been overlooked as well and could be the key to making a more accurate artificial corpse for training HRD dogs.

Figure 1: A) is the structure of pure cadaverine. B) is the structure of pure putrescine.

Derivatization

To derivatize a chemical compound is to change the structure of a compound so it is similar but different from the original compound.[18] This alteration to the amine allows the compound to be more nonpolar, more volatile and allows for the amine to be detected with greater accuracy by the GC-MS.[6] Amines in their natural state are nonvolatile and slightly polar, but with a simple reaction, they can be made more volatile and nonpolar which are the ideal kinds of compounds to be analyzed by the GC-MS allowing for the identification and quantification of more amines in samples.[6]

There are several chemical reactions that can be used to derivatize amines including silylation, acylation, alkylation, halogenation, carbamate formation, sulfonamide formation, phosphonamide formation, Schiff base formation, isothiocyanate and thiourea formation, and chiral derivatization.[6] Each method in turn has numerous derivatizing agents that can be used based on the type of amine and how it is being analyzed.[6] Other methods have been explored previously, which were Schiff base and acylation derivatization for biogenic amines. In this study Schiff base derivatization was performed with trifluoroacytalacetone (FAA) as seen in Figure 2 and can be used on biogenic amines and analyzed primarily with flame ionization detector (FID).[6,7] This method has also been used with gas chromatography- mass spectroscopy (GC-MS) analysis.[6,7] Although this method was used in an article by Khuhawar et al. and were able to detect derivatized forms of putrescine and cadaverine via infrared spectroscopy (IR) and mass spectroscopy (MS) at consistent absorbances and retention times with minimal noise, their results could not be replicated by this lab as seen in Figure 8. [7] Acylation with pentafluoropropionic anhydride (PFPA) (as seen in Figure 3) was used by Narvekar et al.

which yielded consistent and stable derivatized forms of their amines which were detected via GC-MS.[19] This method was employed in the current study but the results showed significant differences compared to what was reported. While not as clean as Narvekar's results, this lab had been able to produce some promising results with this method as seen in Figures 9 and 11. Finally, isobutyl chloroformate (IBC) (see Figure 4) was used in the current study to derivatize amines. The results show the successful derivatization and detection of these derivatives using GC-MS. In Figures 11-21 each of the amine's peak is prominent and has good resolution with its neighboring peaks. The corresponding mass spectra of those peaks are also consistent with their derivatized structure and possible fragmentation.

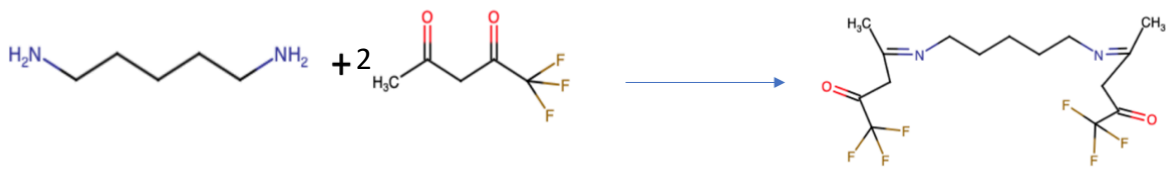

Figure 2: Schiff base reaction of cadaverine with FAA

Figure 3: Acylation derivatization of cadaverine with PFPA

Figure 4: Formation of carbamates of cadaverine with IBC

Decomposition

After death, the decomposition of the human body breaks down compounds such as proteins, lipids, carbohydrates and minerals into simpler compounds.[5] This process starts when the cells of a human body fail to receive oxygen, which triggers an enzymatic self-digestion of the cells.[5] The anaerobic conditions create the optimum conditions for the development of anaerobic bacteria in the intestines and respiratory tract to promote decomposition.[5] The rate of this reaction can be affected by a number of factors such as the age and build of the victim and by various environmental factors like temperature and humidity.[5] Amines such as cadaverine and putrescine are formed from the proteins that are broken down in to peptides.[5] Those peptides are then further broken down into amino acids that is then decarboxylated to form the amines.[5] Some of these decomposition reactions are shown in Figure 5.[20] These amines are responsible for the unique smell that is released during decomposition.[5]

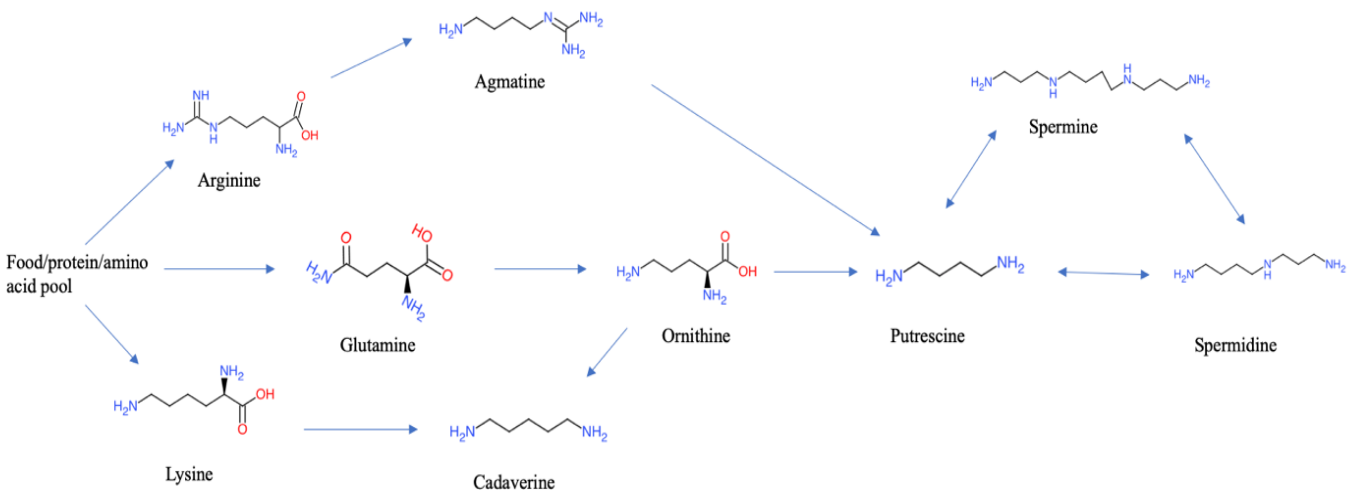

Figure 5: Diagram of the formation of various biogenic amines.[20]

CHAPTER 2: METHODS

Chemicals and Materials

Surrogate Samples

Two samples of clean sand (from Fisher Scientific) were spiked with amines to create surrogate samples. One sand sample was spiked with 5 ml of a 100 ppm solution with both cadaverine and putrescine, which was then evaporated to dryness (S1). The second sample was spiked with 0.005 g of both putrescine and cadaverine dissolved in 400 μL of water and evaporated to dryness (S2).

Grave Soil Samples

Three soil samples were retrieved from the body farm (Forensic Osteology Research Station (FOREST) of Western Carolina University) by an associate. One of the soil samples was collected from an outside area (approximately 20 feet away (uphill) from the body farm) and was used as a reference (S3). The second soil sample was collected from the torso area of a corpse that was placed on 3/17/2021(S4). Third soil sample was collected from the torso area of a body that was placed in the enclosure on 10/15/2020 (S5).

Pseudo Sent Samples

Three pseudo scent products were used: Sigma Pseudo^{TM} Corpse Scent, Formulation I (p4304) (1 ml ampules) (S6), Sigma Pseudo^{TM} Corpse Scent, Formulation II (p3929) (1ml

ampules) (S7), and SOKKS-MPTS ® Cadaver (from Prof. Dr. Wolf A. Kafka Scientific Consulting) (2 tubes) (S8).

sample IDs are in parentheses and are labeled S1-S8

Schiff Base Derivatization

The 3% FAA solution was made by combining 5ml of methanol with 300 μL of FAA (from Aldrich) and was diluted to 10 ml with methanol (from Pharmco-AAPER, HPLC grade). The amine standards were 0.0025 M solutions in ethanol (from AAPER). Buffer was made as a 0.1 M solution of NaOH (10 N solution from Fisher Scientific) in water. Chloroform (from Fisher Scientific) was used as the organic layer to extract the derivatized amines from the aqueous layer. Argon gas was used in place of nitrogen to evaporate the organic layer to concentrate the amines.

Acylation

Amine standards (AS)were made at a concentration of 100 μg/ml in tetrahydrofuran (from Acros Organics). PFPA (from Alfa Aesar) was used as the derivatizing agent. Methanol (AAPER) was used as a solvent.

Formation of Carbamates

Amine standards was created using cadaverine (from Sigma), putrescine (from Aldrich), histamine (from Sigma), and tyramine (from Aldrich) which were dissolved in a 0.1 M HCl solution (from stock room) at 100 ppm. Methanol (from AAPER) and KOH (from Fisher), were combined to saturation to create alkaline methanol. Acetonitrile (from the stock room), toluene (from Aldrich), NaOH (make 2 and 5 M solutions with water as solvent) were used as

dispersive solvents and to aid in stabilize the pH of the reaction, and isobutyl chloroformate (from Aldrich) was used as the derivatizing agent.

Methods

Sample Preparation

5 g of grave soil or surrogated sand sample is combined with 6 M HCl and heated at 100°C for 1-2 hrs. The sample is allowed to cool and is centrifuged at 4400 rpm for 20 minutes. The resulting clear liquid is removed and a small portion is diluted to form the sample that is to be derivatized as seen in the paragraph below. Same procedure was followed for cadaver dog training aids. For liquid cadaver dog training aids (Sigma Pseudo™ Corpse Scent, Formulation I and Sigma Pseudo™ Corpse Scent, Formulation II), 1mL ampule was used for the derivatization and two tubes from the solid training aid (SOKKS-MPTS ® Cadaver) were used for the derivatization.

Then 500 μL of the S1, S3, S4, and S5, 50 μL of S2 was diluted to 5 ml, and 250 μL of S6, S7, and S8 were diluted to 5 ml with 0.1 M HCl. This allowed the sample's signals to fall within the calibration curve, and the concentration of cadaverine and putrescine to be calculated

For the soil samples additional centrifuging was necessary when adjusting the pH. When the precipitate formed, it was centrifuge at 4400 rpm for 10 minutes. The resulting liquid was removed and the pH was adjusted. This was repeated as many times as needed until no more precipitate appeared.

Schiff Base Derivatization

First 2.5 ml of the amine standard was combined with 1.5 ml of FAA solution and 2 ml of buffer. This was heated in a water bath for 15 minutes at 70°C. It was allowed to cool to room

temperature and 3 ml of chloroform was added. The solution was mixed and the chloroform layer was removed. Then 2 ml of chloroform was added and mixed again with the aqueous layer. The chloroform layer was removed and combined with the previous chloroform layer. Finally the chloroform was then evaporated under an argon stream and the residue was redissolved in 200 μL of ethanol before analysis.

Acylation

Several AS (3 ml each) were combined and diluted to 100 ml with tetrahydrofuran to make the amine sample solution. Then 2 ml of the sample solution was combined with 50 μL of PFPA and heated in a water bath for 10 minutes at 60°C. Once at room temperature it was then diluted to 5 ml with methanol and heated again for 10 minutes at 60°C before analyzing.

Formation of carbamates

First 5 mL of the AS or surrogate sample is placed into a centrifuge tube and the pH was adjusted to 12 using the 2M NaOH solution. To this 325 μL of toluene, 1 mL of acetonitrile, and 25 μL of isobutyl chloroformate were added and shaken gently by hand for 5 minutes. The tube was then centrifuged for 4 minutes at 4400 rpm. A portion (150 μL) of the toluene layer was placed into a separate tube and 75 μL of alkaline methanol was added. The tube was mixed by hand for 1minute. Next 450 μL of 5 M NaOH was added and shaken by hand for 1minute. It was then centrifuged for 4 minutes at 4400 rpm. Finally, 1 μL of the toluene layer was then in injected into the GC-MS for analysis. The same method was used on surrogate sand, grave soil, and pseudo scent samples.

Gas Chromatography Mass Spectroscopy Parameters

Schiff Base

A 1 μL of sample was injected into the GC-MS. The oven temperature was set as follows: initial temperature was at 200 °C and then increased to 220 °C at 2°C/minute. The resulting chromatograms and mass spectra were examined for evidence of the derivatized amines.

Acylation

A 1 μL of sample was injected into the GC-MS. The oven temperature was set as follows: 80 °C for 3 minutes, increased to 225 °C at 80 °C/minute and held 225 °C for 3 minutes.

Formation of Carbamates

A 1 μL sample of the final toluene layer was injected into the GC-MS. The oven temperature was set as follows: 100 °C for 1 minute, increased to 160 °C at 10 °C/minute and then increased to 280 °C at 25 °C/minute and held for 14 minutes. The resulting chromatograms and mass spectra were examined for evidence of the derivatized amines.

Calibration Curve

The calibration curves was created for cadaverine and putrescine by plotting concentration in ppm on the x axis and corresponding abundance on the y axis. Solutions of 5, 7, 10, 15, and 20 ppm of cadaverine and putrescine were derivatized, analyzed, and the resulting data was plotted. A linear trendline is fitted and an R^2 value calculated using Microsoft Excel program. The limit of detection (LOD) is found by $LOD = 3.3(Sy/S)$[21]. The limit of

quantification (LOQ) is calculated using LOQ = 10(Sy/S)[21]. Where S is the slope of the line and S_y is the standard deviation of y-intercepts of the regression lines. Uncertainties of sample's concentrations were generated using equation (1).[22]

$$S_x = \frac{S_y}{|m|}\sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y-\bar{y})^2}{m^2 \sum(x_i-\bar{x})^2}} \qquad (1)$$

Where k is the number of measurements made to the unknown sample, n is the number standards used in the calibration curve, m is the slope of the line, y is the average y of the unknown, $\bar{y}$ is the average y from the calibration curve, x_i is the individual concentrations on the calibration curve, and $\bar{x}$ is the mean value of x from the concentrations on the calibration curve.[22] Percent recovery was calculated by dividing the determined concentration of S1 and S2 by the actual concentration of S1 and S2 and multiplied by 100.

CHAPTER 3: RESULTS AND DISSCUSION

Derivatization

The Schiff base method yielded poor results. As seen in Figure 8 there were not any observable amine peaks. This was thought to be because the drying step takes too long and might have evaporated the derivatized amines. In the drying step argon air is used to facilitate the evaporation of chloroform to separate the derivatized amines. The drying method was changed from blowing on the surface of the chloroform solution to submerging the tip of a needle to bubble the argon gas in the chloroform solution. While this new drying method did slightly improve the drying time, no amine peaks were observed.

Derivatized amines were observed with the acylation method (see Figures 9 and 10). However, this method had significant problems. The amine peaks were small compared to the unrelated peaks that also appeared in the chromatogram. These unrelated peaks were present even when no amines were in the reaction mixture. This indicated that the derivatization agent and other compounds in the reaction mixture reacted. Additionally in a sample that had both cadaverine and putrescine in the reaction mixture only a cadaverine peak was visible in the chromatogram (see Figure 9). Several changes to the procedure were made to improve results. This included increasing the concentration of the amines, increasing the amount of derivatization agent and removing the methanol from the procedure but a significant improvement of results was not observed. Therefore a different derivatization method were explored.

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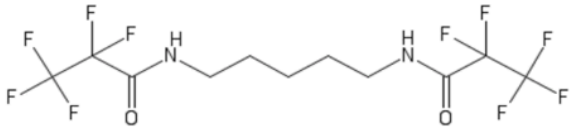
The formation of carbamates with IBC had yielded the best results. In Figures 11-20, one can see not only visible amine peaks but peaks that are prominent and separated from other unrelated peaks in the chromatogram. In Figure 11 where the sample had four different amines present and each one was clearly visible and relatively resolved despite their similar structures and elution times. Different trials were conducted to try and improve the results. In the first trial, the 25 μL of IBC solution was added to the reaction mixture without buffer. Generally, the baseline data was an improvement from the other methods. The samples yielded relatively clean chromatograms with prominent amine peaks (see Figures 13 and 18-21) but the tyramine was not always visible (see Figure 13). Therefore, further trials were conducted to see if certain conditions could result in better data. The next trial increased the amount of IBC to 50 μL to double the amount of derivatization agent. This resulted in even more prominent peaks and, in the case with the sample that had all four amines, tyramine was visible compared to the baseline (see Figure 11). To aid in stabilizing the pH of the reaction mixture a third trial was conducted where 1 mL of a pH 12 phosphate buffer was added right after the pH was adjust to 12 on the samples. This would help keep the pH of the reaction mixture at the optimum pH If looking at figures 14-17 one can see that the unrelated peaks slightly increased in number and intensity.

A)



B)

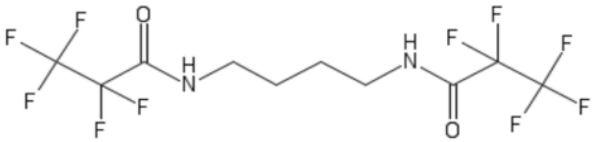
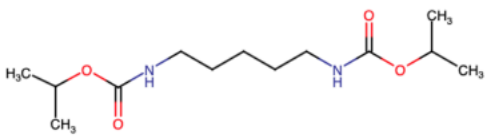


Figure 6: A) structure of acylation derivatized cadaverine with a molar mass of 394.21 g/mol. B) structure of acylation derivatized putrescine with a molar mass of 380.18 g/mol

A)



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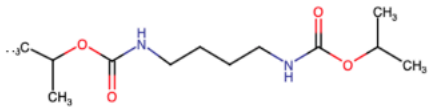
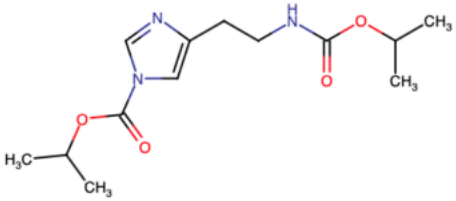


C)



D)

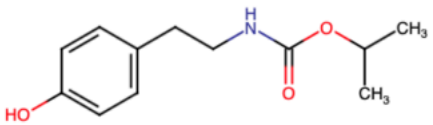


Figure 7: Structures of derivatized amines for the formation of carbamates method. A) cadaverine with a molar mass of 302.39 g/mol, B) putrescine with a molar mass of 288.37 g/mol, C) histamine with a molar mass of 311.18 g/mol, and D) tyramine with a molar mass of 236.29 g/mol

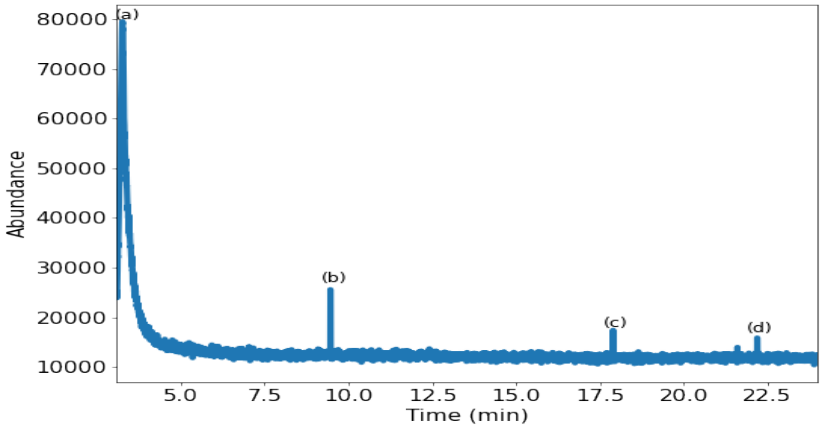


Figure 8: Chromatogram of the derivatized cadaverine using Schiff base procedure. (a) methyl tetradeca-11,12-dienoate (b) 2-pyridinepropanoic acid (c) 4-methyl-2,4-bis(4'-trimethylsilyloxyphenyl) pentent-1 and (d) furan based on the spectral library search

A) ->

B) ->

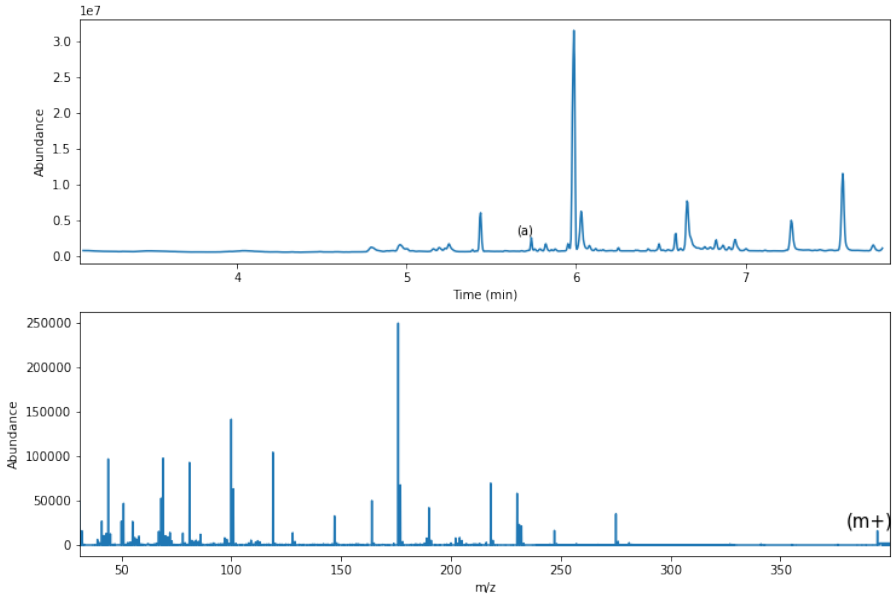


Figure 9: A) The chromatogram of the acylation derivatization of cadaverine and putrescine. The cadaverine peak is labelled (a). Other peaks are unrelated and there is no putrescine peak. B) The mass spectrum of peak at 5.736 minutes of the chromatogram.

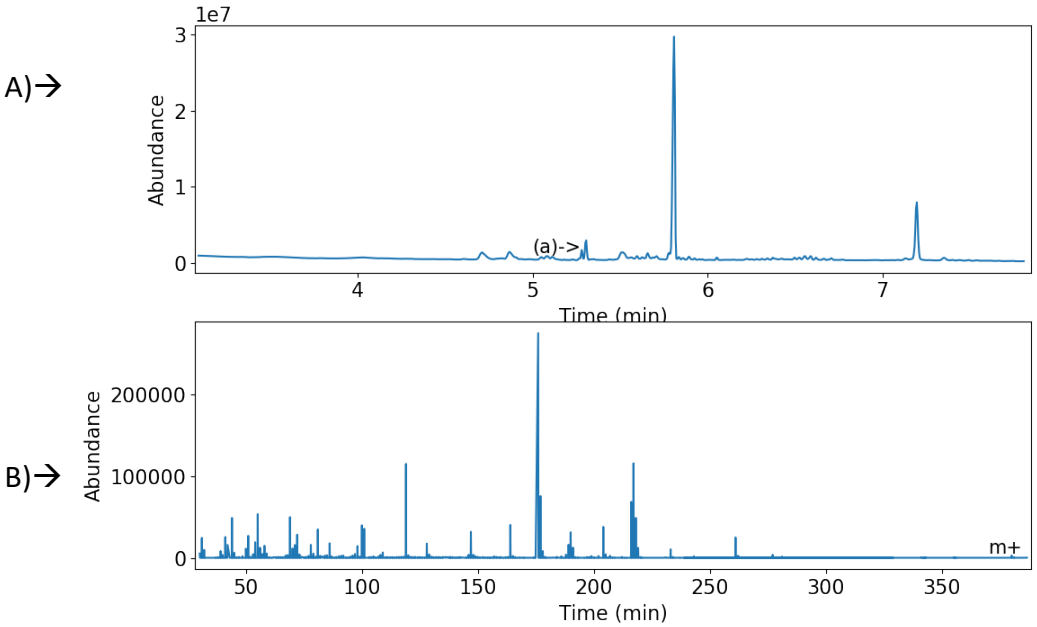


Figure 10: A) The chromatogram of the acylation derivatization of just putrescine. (a) is putrescine. B)the mass spectra of peak at 5.307 minutes of the chromatogram.

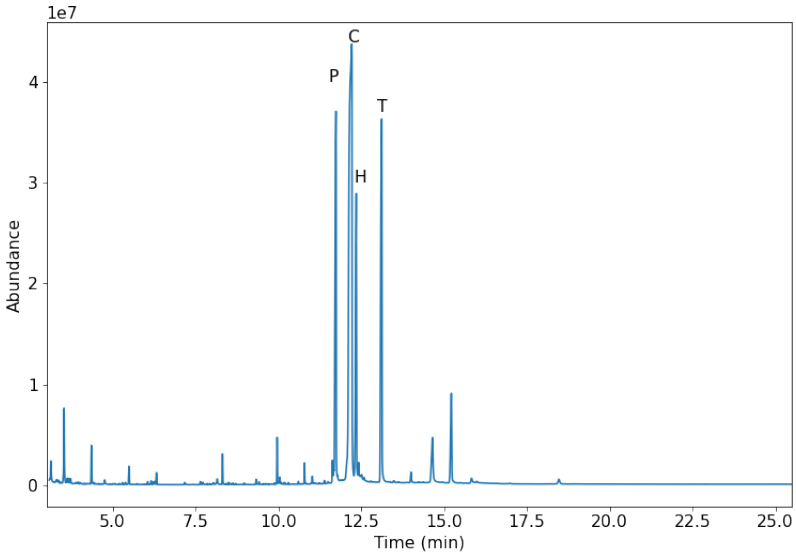


Figure 11: Chromatogram of amines reacted with 50 μL IBC. P is putrescine (retention time is 11.74 min), C is cadaverine (retention time is 12.2 min), H is histamine (retention time is 12.36 min), and T is tyramine (retention time is 13.11).

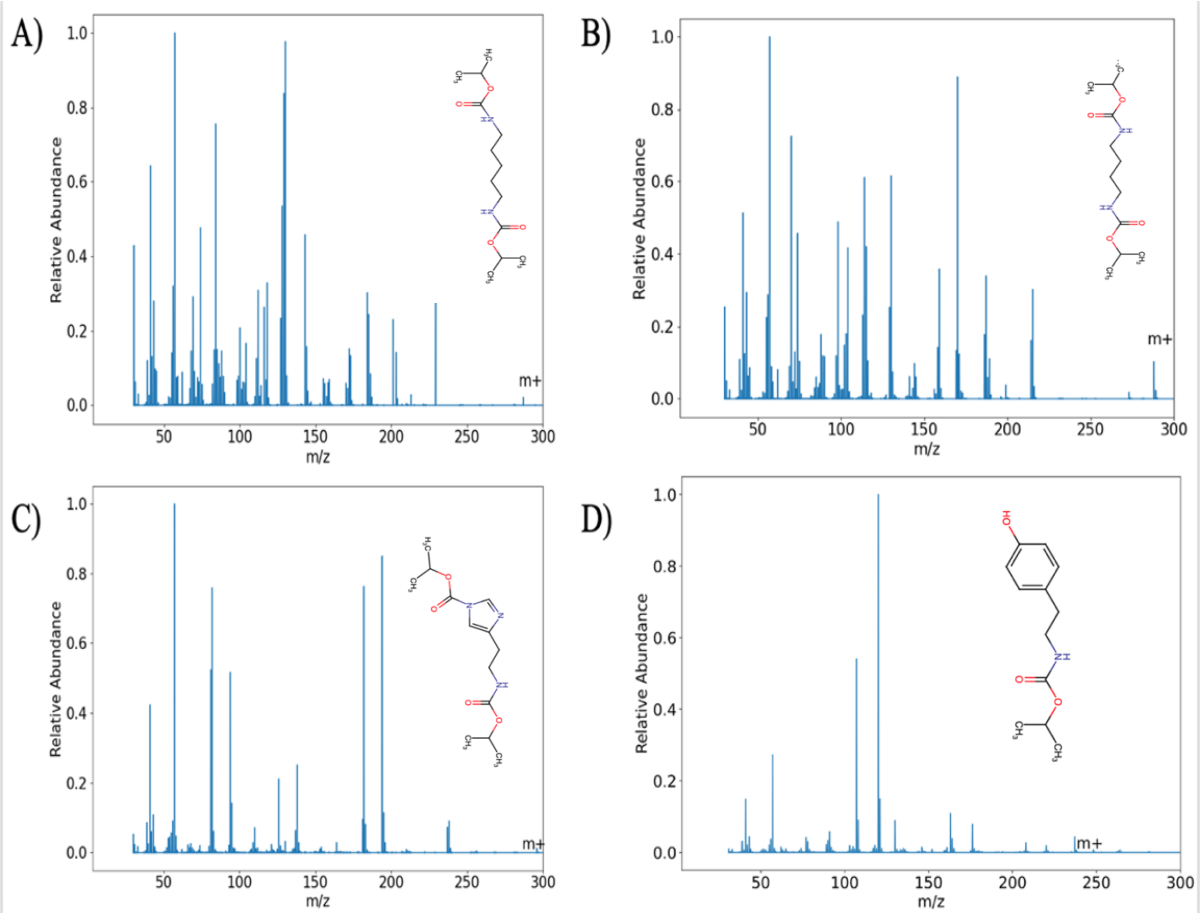


Figure 12: Mass spectra of peaks that are amines derivatized with 50 μL IBC. A) is the mass spectrum of cadaverine, B) is the mass spectrum of putrescine, C) is the mass spectrum of histamine, D) is the mass spectrum of tyramin

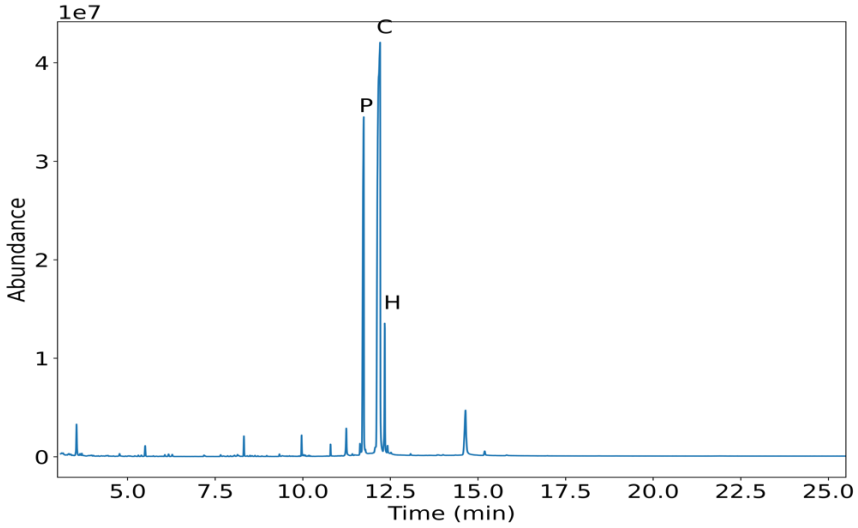


Figure 13: Chromatogram of amines derivatized with 25 μL of IBC. P is putrescine (retention time is 11.74 min), C is cadaverine (retention time is 12.2 min), and H is histamine (retention time is 12.33 min). Tyramine was not visible in this chromatogram.

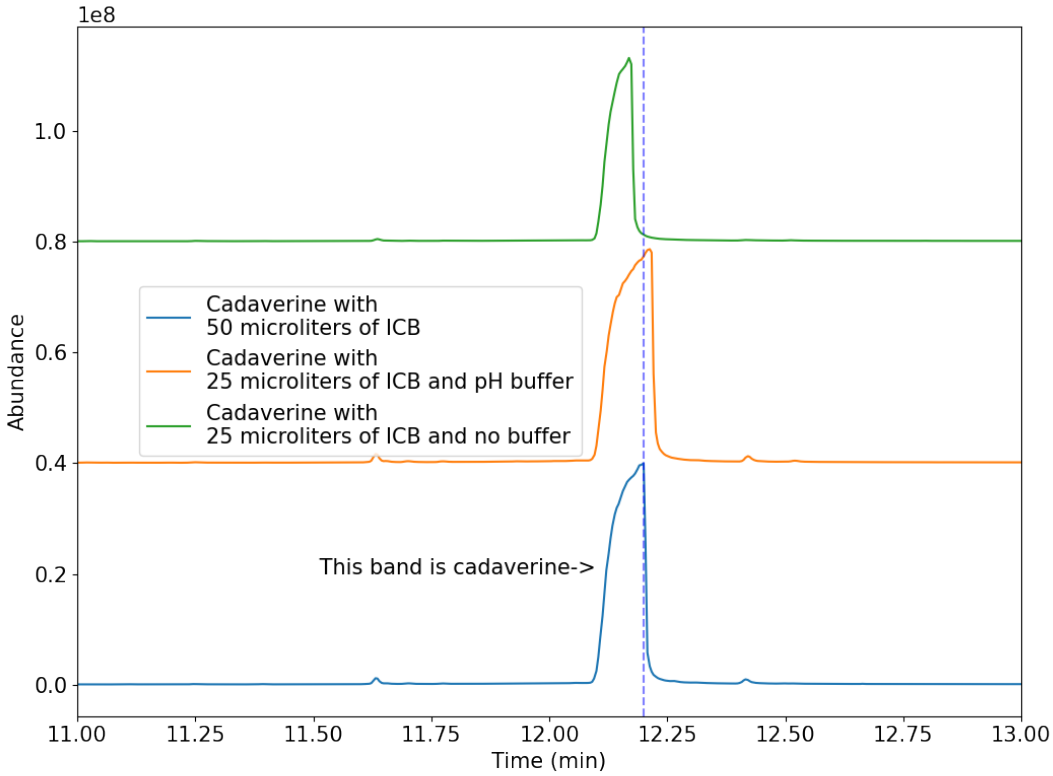


Figure 14: Chromatogram of cadaverine derivatized with 50 μL of ICB (retention time is 12.20 min), with 25 μL ICB and pH buffer (retention time is 12.21 min), and with 25 μL ICB and no buffer (retention time is 12.17 min)

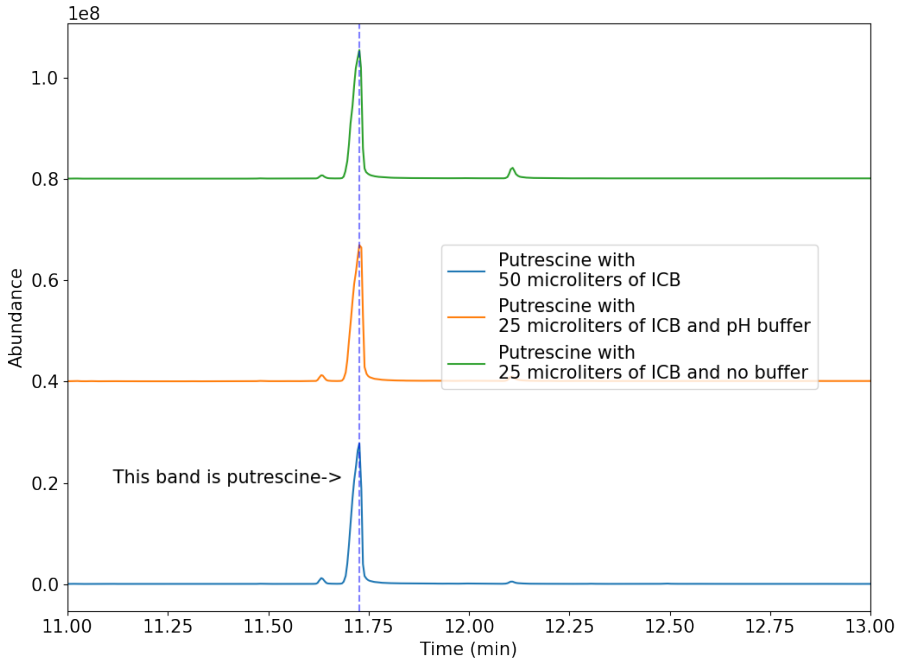


Figure 15: Chromatogram of putrescine derivatized with 50 μL of ICB (retention time is 11.727 min), of AS with just putrescine and pH buffer (retention time 11.727 min), and of AS with just putrescine without any buffer and with 25 μL of ICBF (retention time 11.727 min)

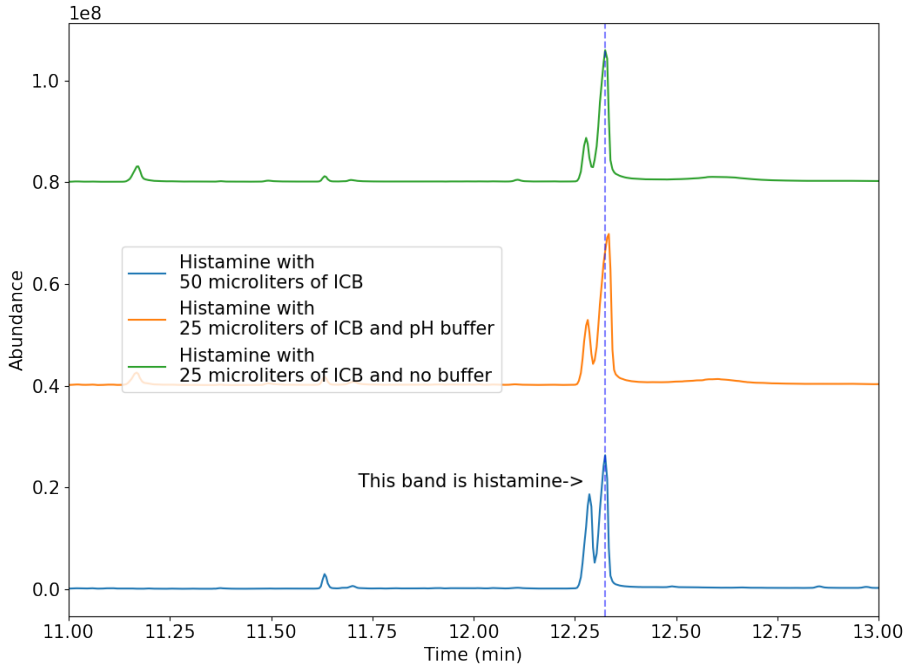


Figure 16: Chromatogram of histamine derivatized with 50 μL of ICB (retention time is 12.325 min), with 25 μL of ICB and pH buffer (retention time is 12.334 min), and without any buffer and with 25 μL of ICBF (retention time is 12.325 min).

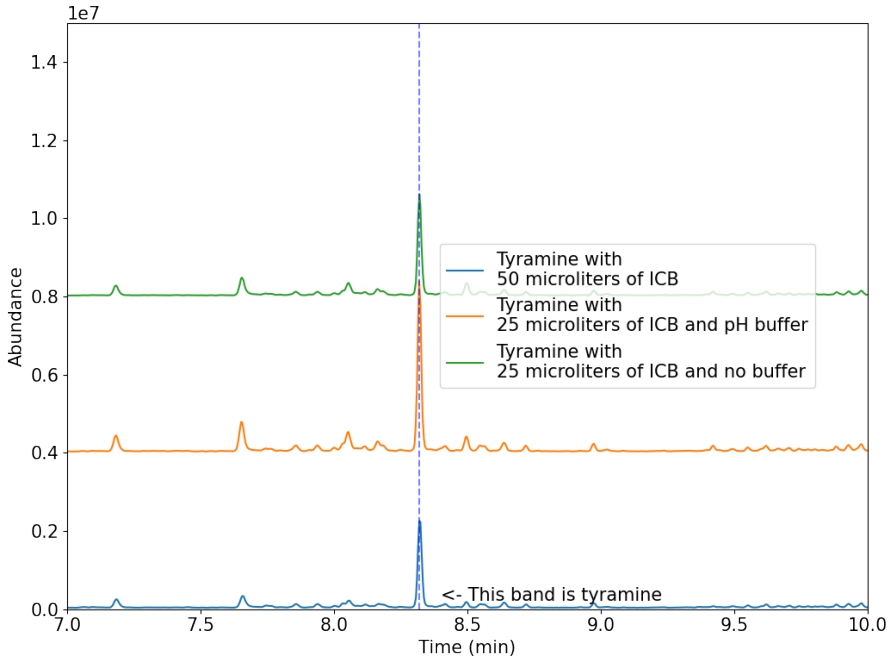


Figure 17: Chromatogram of tyramine derivatized with 50 μL ICB (retention time is 8.32 min), with 25 μL with pH buffer (retention time is 8.31 min), and without any buffer and with 25 μL of ICB (retention time is 8.32 min).

Practical Samples

Only cadaverine and putrescine were selected to be analyzed in all samples because the initial data on grave soil samples and pseudo scents showed that only cadaverine and putrescine were present in these samples. Promising results were gleaned from the various practical samples tested. Amine peaks were prominent in the chromatograms (Figures 22 and 23) of surrogate sand samples. This was especially true for putrescine and cadaverine. The chromatograms of the sand and soil samples were noisier compared to chromatograms obtained from standard amine solutions. The extra peaks were expected given that an unrefined extraction procedure was used to process these samples.

In the grave soil samples cadaverine was the only amine present and is the only amine peak seen in the chromatograms (see Figures 24-26). The highest concentration of amines in the soil samples was with soil that came from the grave of a body that was placed there 10/15/20 (S5). The soil that came from a fresh grave (S4), and the clean soil (S3) were determined to have less amines (see Table 1). This was expected, since this was a fresh grave and the body may not have decomposed enough to have released a significant enough amount of amines compared to the sample of soil from an older grave. A possible reason for the presence of amines in the clean soil sample is that plant material and other organisms that are present in the soil could release amines during the decomposition. The chromatograms of Figures 24-26 also contained several unknown peaks in addition to the derivatized amine peaks which can be attributed to the complex sample matrix and unrefined extraction procedure.

With the pseudo scent products, the results were mixed. Sigma Pseudo™ Corpse Sent Formulation I (P4304) contained cadaverine (see Figure 27). SOKKS-MPTS ® Cadaver didn't show any of the expected amines in the chromatogram (see Figure 29). Sigma Pseudo™ Corpse Sent Formulation (P3929) did have strong putrescine and cadaverine peaks (see figure 28). Since the ingredients and the amounts of these products are a closely guarded secret it is harder to determine why SOKKS didn't show any of the amines that we were expecting. It could be that the concentration of those amines was below the detection limit, or they may have chosen not to use putrescine or cadaverine in this formulation. It also could be that given the nature of the matrix (a Styrofoam like substance) and how it had a tendency to float, the amines residing in the tubes could not be extracted with HCl. Sigma Pseudo™ Corpse Sent samples are significantly

different than the SOKKS training aids. Both Sigma Pseudo™ Corpse Sent samples were in liquid form and thus easier to process. SOKKS training aids arrived as scent embedded solid polymeric tubes, and the manufacturer states that all the compounds are present in very low concentrations.

Only the sand samples, S1 and S2, have a known concentrations of amines and can potentially aid in the validity of the calibration curves. S1 had a 76% recovery of cadaverine and 85% recovery of putrescine (see Table 1). S2 had a 140% recovery of cadaverine and 110% recovery of putrescine (see Table 1). This indicates that the calibration curves were not as accurate as they could be or that the extractions were not as effective they could be. This can be due to a variety of reasons. The diluted concentrations of the sand samples used was close to their corresponding LOQ, which would limit the reliability of the trendline in calculating the concentrations. The abundance of the amine peaks could have also been inflated by other signals that were detected at the same retention time. Many problems of the calibration curve and the determined concentrations could be alleviated or reduce with further experimentation given the results of the various trials conducted. Using an internal standard would likely make for a more accurate and linear calibration curve, the extraction procedure can be refined to remove more unrelated compounds from these complex sample matrices, and the GC-MS parameters could be optimized to maximize resolution of all peaks.

Table 1: Table of signals of amines from samples S1-S8 which were used to calculate the concentration of amines in the original samples.

Sample ID	Sample description	Concentration of undiluted sample of cadaverine (ppm)	Concentration of undiluted sample of putrescine (ppm)	Percent recovery cadaverine	Percent recovery putrecine
S1	Surrogate sand: 5 mL of 100 ppm standard in 5 g of sand	76 ± 1	85 ± 1	76%	85%
S2	Surrogate sand: 0.005 g of amines in 5 g of sand	1400 ±1	1100 ± 1	140%	110%
S3	Soil sample collected outside the body farm	37 ± 1	NA	NA	NA
S4	Soil sample collected from the torso area of a corpse placed on 3/17/2021. The samples was collected on 3/18/2021	31 ± 1	NA	NA	NA
S5	Soil sample collected near to torso area of a corpse placed on 10/15/2020. The samples was collected on 3/18/2021	33 ± 1	NA	NA	NA
S6	Sigma Pseudo™ Corpse Scent, Formulation I (p4304)	66 ± 1	NA	NA	NA
S7	Sigma Pseudo™ Corpse Scent, Formulation II (p3929)	130 ± 1	240 ± 1	NA	NA
S8	SOKKS-MPTS ® Cadaver	NA	NA	NA	NA

Table 2: Concentrations of cadaverine and putrescine in psudo corpse samples in mg/mL with the associated error.

Cadaver dog training aid	Concentration of cadaverine mg/mL	Concentration of putrescine mg/mL
S6: Sigma Pseudo™ Corpse Scent, Formulation I (p4304)	426 ± 6	Not detected
S7: Sigma Pseudo™ Corpse Scent, Formulation II (p3929)	$780. \pm 6$	$1440. \pm 6$
S8: SOKKS-MPTS ® Cadaver	Not detected	Not detected

Table 3: Concentrations of cadaverine and putrecine of grave soil samples in mg/mL with associated error

Soil sample	Amount of cadaverine in soil mg/g	Amount of putrescine in soil mg/g
Reference soil sample	31 ± 1	Not detected
Recent corpse soil sample	33 ± 1	Not detected
Old corpse soil sample	37 ± 1	Not detected

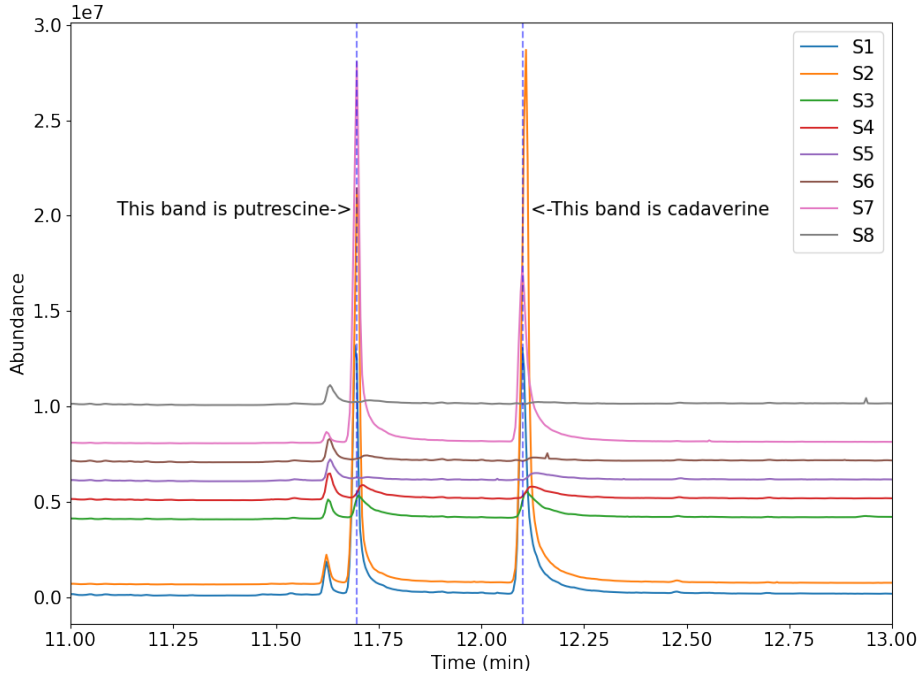


Figure 18: Chromatogram of S1-S8.

Table 4: The retention time of the putrescine and cadaverine peaks of samples S1-S8

Sample names	Retention time of Putrescine (min)	Retention time of Cadaverine (min)
S1: sand spiked with amine solution	11.697	12.104
S2: sand spiked with amines in water	11.701	12.109
S3: clean soil sample	NA	12.126
S4: soil sample from a new grave	NA	12.134
S5: soil sample from an old grave	NA	12.113
S6: pseudo scent (P4304)	NA	12.161
S7: pseudo scent (P3929)	11.697	12.1
S8: SOKKS	NA	NA

Calibration Curve

Calibration curves were made for putrescine and cadaverine, which were the two most prominent peaks and were the most resolved from each other. Figure 31 is the putrescine calibration curve and Figure 30 is the cadaverine calibration curve. Each figure has their own corresponding linear equation, R^2 value, LOD and LOQ values. The R^2 values of both calibration curves were not satisfactory. Due to time constrains these calibration curves were used for further analysis without improvements. A higher quality calibration curve could be obtained also with the use of an internal standard such as 1,7-Diaminoheptane or N,N-Bis-(3-aminopropyl)methylamine.

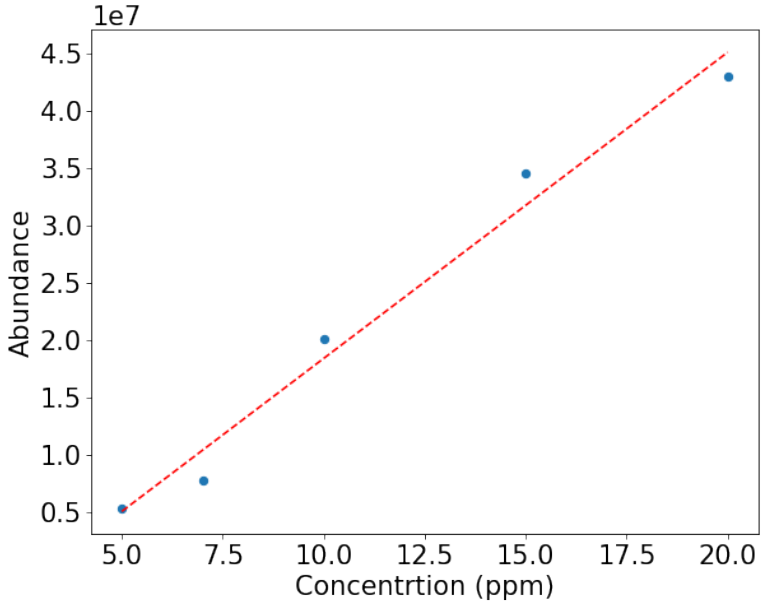


Figure 19: Calibration curve of cadaverine at concentrations of 5, 7, 10, 15, and 20 ppm. The trendline equation is y= 2666063.1x -8254897.8 with R^2= 0.97914916. LOD= 3 ppm and LOQ= 10 ppm.

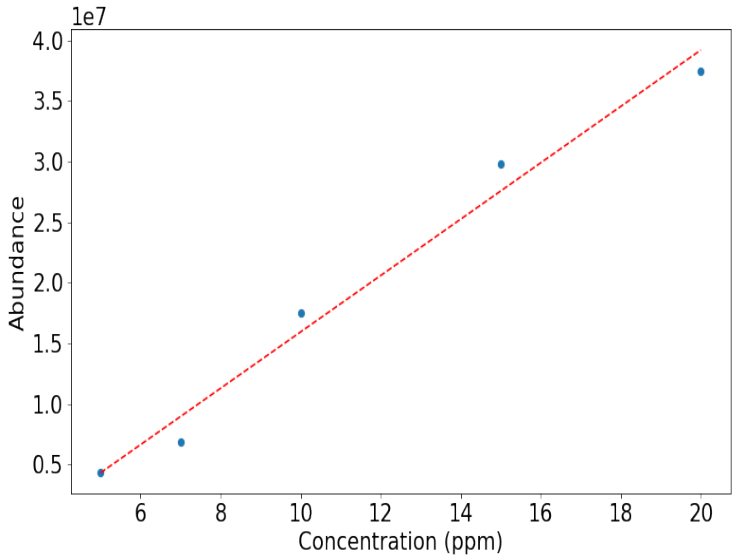


Figure 20: Calibration curve of putrescine at concentrations 5, 7, 10, 15, and 20 ppm. The trendline equation is y= 2324858.49x -7289632.2 with R^2= 0.98196165. LOD= 3 ppm and LOQ= 10 ppm.

CHAPTER 4: CONCLUSION AND FUTURE DIRECTIONS

In general, the formation of carbamates derivatization method is relatively quick and reliable. From stock solutions the amines that are present are easily detected by the GC-MS and produce relatively clean chromatograms. This is true whether the amines are combined into one solution or separated in different solutions. The calibration curve is relatively linear and accurate given the lack of more refined extraction procedure for the practical samples and not using an internal standard. The developed method shows promise in the analysis of amines in the grave soil and cadaver dog training aids.

Elevated concentration of cadaverine was observed in the soil samples obtained from the old corpse (7 months) compared to the grave soil obtained from the new corpse (1 day) and the reference soil sample obtained outside of the body farm. Interestingly, putrescine was not detected in any of the soil samples. This may be due to the presence of putrescine in the soil at below the detection limit of this method This method could also aid in analyzing the pseudo scent products and in comparing their amine composition to that of grave soil. Cadaverine was present in both Pseudo™ Corpse Scent, Formulation I (p4304) and Sigma Pseudo™ Corpse Scent, Formulation II (p3929). This could be an indication that these two cadaver dog training aids mimic different stages of decomposition. Interestingly, cadaverine or putrescine were not present in SOKKS-MPTS ® Cadaver training aid samples. This could be due to the problems with the sample matric or the presence of amines at a lower concentration than the detection limit of this method.

While this method is a step in the right direction in achieving the goal of more accurate training aids for cadaver dogs through the analysis of grave soil there can always be improvement. Tyramine and histamine have a tendency to elute from the GC column relatively close to each other. The GC parameters could be optimized to maximize the resolution between amine peaks. The amount of derivatization agent could also be optimized. Tyramine had a much more prominent peak when more agent was used, meaning that there may not be enough derivatizing agent for the amount of amines used. Additionally, the extraction procedure could also be improved to minimize the number of extra compounds that are extracted with the amines to aid in cleaning up the chromatogram.

Ideally multiple surrogate samples, grave soil samples and pseudo corpse samples should be analyzed to determine the percent recovery and reproducibility of this method but due to the COVID-19 pandemic it was not possible to perform this task. Despite these flaws this derivatizing and sample processing methods developed are reliable and hold much promise in analyzing grave soil for the improvement of cadaver dogs training aid.

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