APPROVAL SHEET

Title of Dissertation: Molecular Ionization Desorption Analysis Source for Mass Spectrometry: Food Authenticity and Other Applications

Name of Candidate: Ciara N. Pitman
Doctor of Philosophy, 2022

Dissertation and Abstract Approved:_____
Dr. William R. LaCourse
Professor
Department of Chemistry and Biochemistry
University of Maryland, Baltimore County

Date Approved: _____

ABSTRACT

Title of Document: MOLECULAR IONIZATION DESORPTION ANALYSIS SOURCE FOR MASS SPECTROMETRY: FOOD AUTHENTICITY AND OTHER APPLICATIONS

Ciara Nicole Pitman, Ph.D., 2022.

Directed By: Professor, Dr. William R. LaCourse, Department of Chemistry and Biochemistry

Molecular Ionization Desorption Analysis Source (MIDAS) is a powerful tool for rapid food authenticity and adulteration detection. The goal of this research is to investigate the versatility of the MIDAS as an analytical tool for several applications with the focus being on food adulteration detection and authenticity. MIDAS followed by Time-of-Flight mass spectrometry was used to authenticate commonly adulterated foods (i.e., vanilla extracts, vanilla beans, and black peppercorns). The analysis of vanilla beans and black peppercorns spurred the creation of additional sample platforms to secure solid samples under the gas stream during analysis. In addition, the applications highlighted the versatility of the MIDAS to be used for liquids, pastes, and solids. In addition to food analysis, the MIDAS has the potential of being utilized in the forensic field. Rapid detection techniques such as chromatographic and other ambient ionization sources that have been used in the food field overlap with those seen in the forensic field. Metabolites of THC and nicotine in synthetic earwax was conducted to further demonstrate the versatility of the MIDAS

as an ionization source. Cotton swabs were introduced as a sample holder for earwax analysis. MIDAS is a rapid, powerful, and flexible analytical tool that can be applied to vast array of sample types (i.e., powders, pastes, solids, waxes) and therefore would be advantageous to many fields of study.

MOLECULAR IONIZATION DESORPTION ANALYSIS SOURCE FOR MASS SPECTROMETRY: FOOD AUTHENTICITY AND OTHER APPLICATIONS.

By

Ciara Nicole Pitman.

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore County, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2022

Dedication

I dedicate this dissertation to my parents, Jim and Julia, and my sister, Mackenzie. Mom and dad, your unconditional love, support, and sacrifice made this all possible. You always encouraged me to go after my dreams and did everything possible to make them come true. When things got tough you were always there with big hugs and plenty of tacos. To my sister and biggest cheerleader, thank you for always being my number one fan and showing me that even when things get tough there is a reason to smile.

Acknowledgements

There are numerous people to acknowledge and thank for joining and assisting me on this journey. I would like to first thank my advisor Dr. William LaCourse. Thank you for providing a learning environment that was fun, challenging, and mentally stimulating. Not only did you guide me through the graduate process, but you also taught me many life lessons. With thought provoking questions, cool fun facts, and constant laughs you challenged me to think outside of the box with both research and life. I would not be the scientist I am today without your support and mentorship.

Next, I would like to sincerely thank my committee members Dr. Chengpeng Chen, Dr. Brian Cullum, Dr. Marie-Christine Daniel-Onuta, and Dr. Upal Ghosh. Thank you all for assisting me in this journey and challenging me to be the best scientist I could be. I have enjoyed my time learning invaluable lessons from each of you.

I would like to thank all my lab mates past and present. Amanda Belunis and Kayry Segarra thank you for your support, friendship, laughs, and copious amounts of snacks. I would like to also thank Maggie LaCourse for always being there to help with paper edits and countless practice presentations. You were always there for a good science talk, a much-needed walk, and a supply of tacos for those late nights.

I would like to thank all the wonderful people I have met at UMBC. To my dear friends Chanda Lowrance, Curtis Jones, and Dan Kazal thank you for always being there for a pick me up, a good laugh, and a willingness to sit through practice presentations. I would like to also thank Evgenia Barannikova for being my guide and role model throughout the years.

Thank you to all the chemistry department faculty and staff that have assisted me throughout the years. I would like to give a special thanks to Frank Tyminski, Ramona Patel, and Patty Gagne for allowing me to ask and being willing to answer my many questions.

My support system outside of UMBC is incredible and I could not have done this without you. I would like to thank my childhood, high school, and college friends who stuck it out with me. Thank you for always being supportive and loving me through all the busy times. I want to extend a huge thank you to my Baltimore Martial Arts Academy family. The love and support you have showed me is indescribable. I am extremely blessed to know such amazing souls.

Lastly, but certainly not least, I would like to say thank you to my family. A special thank you to both of my grandmothers, Linda Cross and Sophie Pitman, for the constant love and support. Thank you to my great aunt, Katherine Prodanovitch, for always encouraging me in my educational pursuit. My parents and my sister, for whom this dissertation is dedicated, without you none of this would be possible. This is just as much as your accomplishment as it is mine. I love you more than words can express.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction to Food Authenticity and Adulteration Methods

1.1. Spices and Flavorings

Defined by the U.S. Code of Federal Regulations a spice is “any aromatic vegetable substance in the whole, broken, or ground form, except those substances which have been traditionally regarded as foods, such as onions, garlic, and celery; whose significant function in food is seasoning rather than nutritional; that is true to name; and from which no portion of any volatile oil or other flavoring principle has been removed”[1]. Spices and herbs have been utilized for centuries for flavoring foods and beverages, they have been used for medicinal purposes, and even for pigments. Over the years the increase interest of using spices for health, food, and cosmetic products has led to the increased interest and desire for quality spice products[2, 3]. Due to high demand and economical constrictions, the likelihood of spices being adulterated with inferior products has increased[4, 5]. Additives are common when there is a need to cut production cost and increase revenue. These adulterants decrease the value of the spice products and in some cases can pose health risks[4, 6].

According to the Food and Drug Association (FDA), under the Federal Food, Drug, and Cosmetic Act (FD&C Act) subchapter IV, food is considered adulterated if it contains any substance that is hazardous to health, contains any substance that has been added, abstracted, or substituted, or has any color that is added and considered harmful[1]. Risk of adulteration is higher with products that are in high demand or

those that have limited availability. One usual form of adulteration is the addition of dyes to enhance the color of the spice[2, 5]. This gives the illusion of the spice being fresher and therefore more appealing to the consumer. Another example is the addition of chemicals that have the same smell or taste as the natural spice compounds[2]. Additives like this help give the illusion of the product being fresher or have a higher concentration of the natural spice. Lastly, another common additive is the addition of substances that mimic that of the main spice[2]. This can be either another lesser spice, seed, or object that has a similar shape, size, and color. Due to increased risk of adulteration, there is a need for rapid detection methods. Quick detection and authentication during the quality control process would be advantageous to both manufacturer and consumer.

1.2. Vanilla and Peppercorn Detection

Two of the most targeted spices/flavorings are vanilla and black peppercorns. Vanilla is one of the most common flavorings to use in food and beverage products[7-13]. With the public's tastes shifting towards all-natural products, costliness of harvesting vanilla, and a range of other market forces, vanilla extract has become a target of adulteration[8, 11-17]. Enhancers are added to extracts to create a cheaper product or the origin of the beans/extract are not properly disclosed, creating a misleading purchase for the consumer. In addition to cost, another concern is the presence of harmful additives such as coumarin which may be found in vanilla extracts imported from other countries[11, 13, 18]. Coumarin addition to food products as a means of enhancing flavor and fragrance has been banned by the United States' FDA due to its hepatoxicity, but it is still used in other countries such as Mexico and the European

Union[8, 11, 13, 16, 18, 19]. Therefore, techniques for quality control are needed to screen for potential breaches of authenticity.

Black pepper or *Piper nigrum (P. nigrum)* is valued for medical use, food flavoring and preservation, as well as an ingredient in perfumes[20, 21]. Some common adulterants of black peppercorns are papaya seeds, mineral oil, other strands of pepper, other seeds and berries, spent black pepper, and stems and chaff of black pepper[2, 6, 22]. Black pepper powders are commonly adulterated with dyes, papaya seed powder, other seed and berry powders, starch, monosodium glutamate, other parts of the berry, and chili powder[2, 6]. The most common additive is the papaya seed due the visual similarities. Rapid detection methods are needed to ensure product quality.

1.3. Current Methods of Detection

1.3.1. Vanilla

Some of the current techniques for authentication of vanilla extracts include those of chromatographic and ambient ionization techniques (Table 1.1)[7-9, 11-13, 15-19, 23]. High Performance Liquid Chromatography (HPLC) with mass spectrometry (MS) and electrochemical detection and Gas Chromatography (GC) with MS have been utilized for quantitative and qualitative analysis of vanilla[23]. Chromatography is a popular analytical technique for detection and quantitation. GC with mass spectrometry has been used to identify coumarin in vanilla samples and to identify key odorants in Tahitian vanilla beans[10]. Coumarin was identified in Mexican vanilla samples by GC-MS in under five minutes. This method was also used to

successfully distinguish and detect the key odorants associated with Tahitian vanilla beans[10]. GC techniques are ideal for identifying and quantifying volatile compounds, but the technique is limited to just volatile samples with some non-volatile exceptions.

HPLC techniques have the capability to separate compounds based on polarity, size, and charge. HPLC has been paired with different detectors to identify and quantitate vanilla compounds in extracts. The most common detector is mass spectrometry because of its ability to be used for complex matrixes and it is selective and sensitive[7]. UV detectors can be used in conjunction with mass spectrometry to better identify compounds. Another detector is an electrochemical detector (ECD) and has been used for detecting guaiacol content in vanilla extracts which is commonly analyzed by GC[13, 17]. This method is more effective because ECD is more sensitive than UV and does not require the extra extraction step that is seen in GC for sample preparation. HPLC techniques can analyze a variety of sample types and is ideal for quantitation. However, all chromatography methods have lengthy run times and extensive sample preparation that put them at a disadvantage.

Table 1.1. Chromatographic and ambient ionization techniques for vanilla authenticity.

Technique	Analytes detected
HPLC with Mass Spectrometry	Vanillic acid, isovanillin, vanillin, o-vanillin, ethyl vanillin, coumarin
GC with Mass Spectrometry	Vanillin, coumarin, anisaldehyde, guaiacol, 3-methylbutanoic acid, 2-methylbutanoic acid, p-cresol, ethyl (E)-cinnamate, methyl (E)-cinnamate, eugenol, anisyl alcohol, phenylacetic acid, 3-phenylpropanoic acid, isovanillin, anethole, 2-mehtylpentanoic acid, 1,2-dimethoxybenzene, (2E,4E)-deca-2,4-dienal
HPLC-UV- Mass spectrometry	Vanillin, ethyl vanillin, coumarin, 3,4-(Methylenedioxy)acetophone
HPLC with Electrochemical Detection	Vanillin, guaiacol
Atmospheric Solids Analysis Probe	Vanillin, vanillic acid, hydroxybenzaldehyde, ethyl vanillin, coumarin
Direct Sample Analysis	Vanillin, coumarin,4-hydroxybenzaldehyde, benzoic acid

Overcoming the lengthy run times and sample preparation procedures, techniques such as ambient ionization were utilized for rapid detection and characterization. Ambient ionization is defined by the ability to produce a mass spectrum from a surface with little to no sample preparation and has been used as a high throughput method for several research fields[24]. One of the fields that has utilized the high throughput method is the food fraud and authentication field. Vanilla adulteration and compound characterization has been growing in the ambient field. Previously published work with vanilla has utilized Atmospheric Solids Analysis Probe (ASAP) and Direct Sample Analysis (DSA) for rapid detection of adulterants in vanilla extracts and vanilla food products[9, 11, 25]. Both techniques have a similar ionization method to tradition Atmospheric Pressure Chemical Ionization (APCI).

ASAP (Figure 1.1) uses a probe to house the sample for direct analysis and can be used to test both solid and liquid samples[9]. ASAP has been used to determine the presence of vanilla compounds in food products such as extracts, cookies, and coffee[9]. The downfall of ASAP is the need to change or clean the probe in between samples which decreases the high throughput of the method.

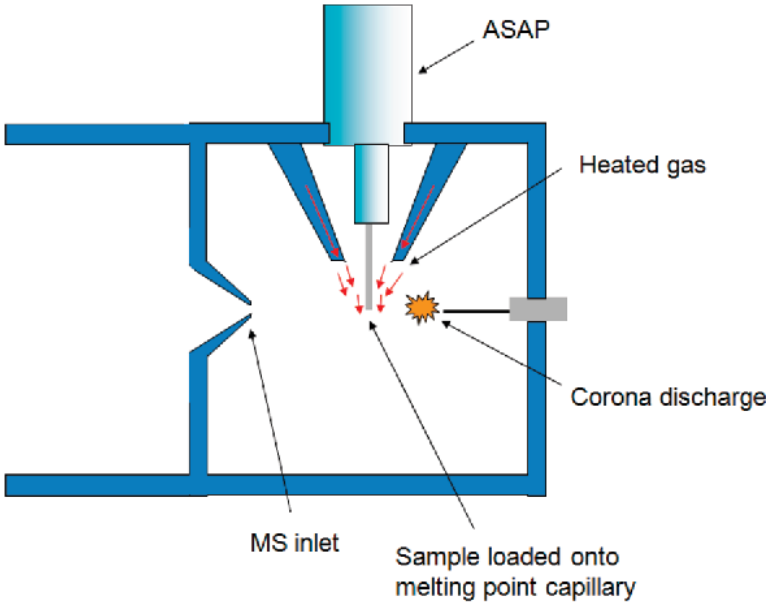


Figure 1.1. Schematic of Atmospheric Pressure Chemical Ionization (ASAP) [24].

DSA has a higher throughput capability than ASAP and has been used to rapidly characterize vanilla extracts. The DSA (Figure 1.2) can test 13-spots consecutively with its built-in sample holder. Using the DSA, vanilla extracts and tonka bean extracts were compared to display the differences in spectra as well as the differences of pure and imitation vanilla extracts[11, 16]. The disadvantage of the DSA is that it is limited to only 13-spots and then the sample holder needs to be changed. Also, due to its "fixed" configuration large, irregular shaped, and some powdered samples cannot be assayed using the DSA. A method with more sample positions and

flexibility of source position is needed to create a higher throughput method. Rapid characterization of vanilla samples will aid in identification of additives and confirm authenticity before the product is purchased. This will prevent the customer from purchasing fraudulent and potentially toxic products. A high throughput method for characterizing vanilla samples will be discussed in later sections.

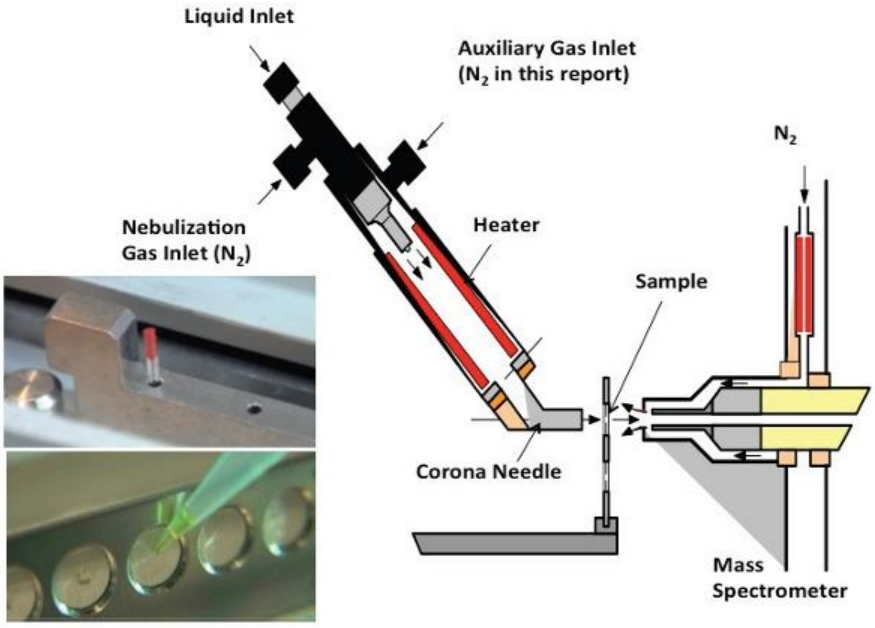


Figure 1.2. Schematic of Direct Samples Analysis (DSA) [11].

1.3.2. Black Pepper (Piper Nigrum)

Methods for analyzing black pepper compounds are like those of vanilla. Some methods for detection of adulteration and characterization of black pepper include chromatographic techniques and more recently ambient ionization[20, 26-29]. Thin Layer Chromatography (TLC), HPLC, and GC methods have been utilized to explore black pepper. High Performance TLC (HPTLC) has been used to fingerprint piperine in pepper and is usually used in conjunction with chromatographic methods[21]. Liquid Chromatography is one of the more commonly used methods for pepper

analysis. HPLC with PDA detection and LCMS methods have also been used to identify structural and sensory compounds, verify quality, distinguish differences between pepper and papaya, and estimate concentrations of piperine in black pepper[20, 30]. GCMS has been used for similar methods and is commonly found accompanying LC methods. GCMS methods have been predominantly used for analysis of essential oils and other oil based samples[29]. As stated above chromatographic techniques have their limitations and can inhibit throughput.

Table 1.2. Chromatographic and ambient ionization techniques for black pepper analysis.

Technique	Analytes detected
HPTLC /TLC	Piperine
LCMS	Piperine, piperyline, piperloguminine, piperttline, piperoleine, dihydropipernonaline, retorfractamide, piperroleine, guineensine, piperonol, caryophyllene, carpaine, glucotropaeolin
HPLC	Piperine, piperyline, piperloguminine, piperttline, piperoleine, dihydropipernonaline, retorfractamide, piperroleine, guineensine, piperonol, caryophyllene, carpaine, glucotropaeolin
GC-MS	Piperine, piperyline, piperloguminine, piperttline, piperoleine, dihydropipernonaline, retorfractamide, piperroleine, guineensine, piperonol, caryophyllene, carpaine, glucotropaeolin
DART	Piperine, capsaicin, curcumin, coumarin, cinnamaldehyde, fruit, leaf, and root of P. nigrum, P. chaba, and P. longum, Piperine, piperyline, piperloguminine, piperttline, piperoleine, dihydropipernonaline, retorfractamide, piperroleine, guineensine, piperonol, caryophyllene, carpaine, glucotropaeolin

The most prevalent ambient ionization source used for black pepper analysis is Direct Analysis in Real Time (DART). DART is a plasma-based ionization source and has similarities to that of ASAP and DSA. DART, Figure 1.3, has been used to distinguish black pepper from other spices, identify major compounds in pepper samples, and identify differences between pepper species[20, 26, 31]. DART with principal components analysis (PCA) was successful in the differentiating black

pepper from other spices in four different brands of spices[20]. A similar method was also successful in differentiating between different pepper species and determining the differences between the fruit, leaf, and root of each species[31]. DART is rapid detection and characterization method but has disadvantages. Like ASAP one sample at a time can be tested which hinders the throughput of the technique. It also is more expensive than ASAP and DSA due to the use of helium gas[32]. There are options to use nitrogen as the substitute ionization gas, but literature suggest that the signal and sensitivity suffers when nitrogen is used[32]. Ambient ionization is a rapid method to characterize and authenticate samples. Current methods have some limitations that hinder high throughput and sensitivity. The Molecular Ionization Desorption Analysis Source (MIDAS) overcomes those barriers and provides a rapid and flexible method[33].

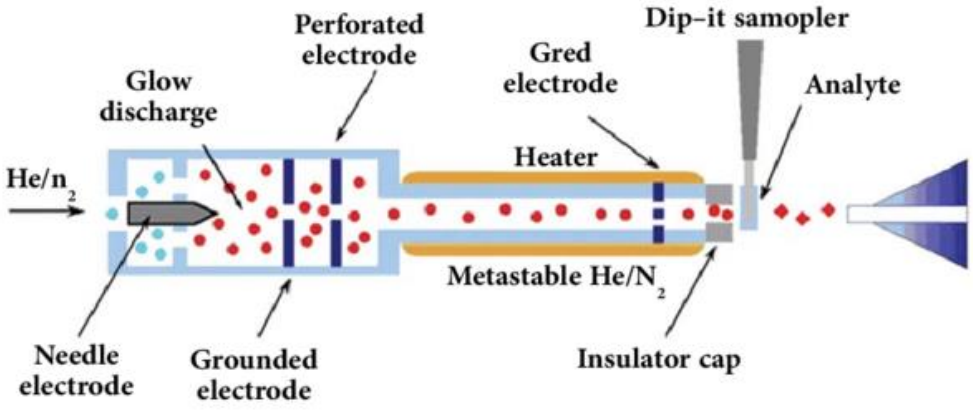


Figure 1.3. Schematic of Direct Analysis in Real Time (DART) [24].

1.4. Introduction to the Molecular Ionization Desorption Analysis Source

The Molecular Ionization Desorption Analysis Source (MIDAS) is a desorption atmospheric pressure chemical ionization (DAPCI) source constructed in 2016 by Winter *et al*[33, 34]. The source was constructed with a similar design to PerkinElmer's Direct Sample Analysis (DSA), but with modifications to accommodate more sample types. These modifications included a source control to adjust the source in the XYZθ direction and a moving sample plate holder in the XY direction. The sample plate holder makes it possible to have interchangeable sample plates and therefore the MIDAS can accommodate several sample types and shapes.

The body of the MIDAS (Figure 1.4) consists of a gas heater that is made out of a stainless steel tube with stainless steel ball bearings to increase heat transfer[33, 34]. The nitrogen is supplied by an in-house nitrogen source that flows through the tube and over the corona pin, fastened to the end of the heater tube. The tube is wrapped with heating tape that is connected to a variable power supply which allows the temperature to be adjusted as needed. MIDAS uses a corona pin for the corona discharge with a high voltage line directly connected to the pin. Unlike the DSA, the cylinder that the pin sits in has external threads for the use of attaching nozzles. The most used nozzle was the 15mm long glass nozzle with an inner diameter of 1.5mm[33]. The entire source is held together by a source pitch and XYZ control stage. This allows the source to be moved in the XYZθ direction to better optimize distance and angle for sample analysis. Attached to the source control is a camera that allows for remote visuals of sampling with either picture or a live video. Next to the camera are lights that are used to provide better lighting.

In contrast to DSA, the sample platform is planar, and the nozzle sits at angle of 40º to 55º from the platform[33]. In addition to the platform being planar, it sits on a XY moving stage that is powered by a 24V DC power supply and is controlled through the 2.11 version of Immediate Motion Creator software[33]. The platform allows for interchangeable sampling plates to be used which can increase the throughput of analysis.

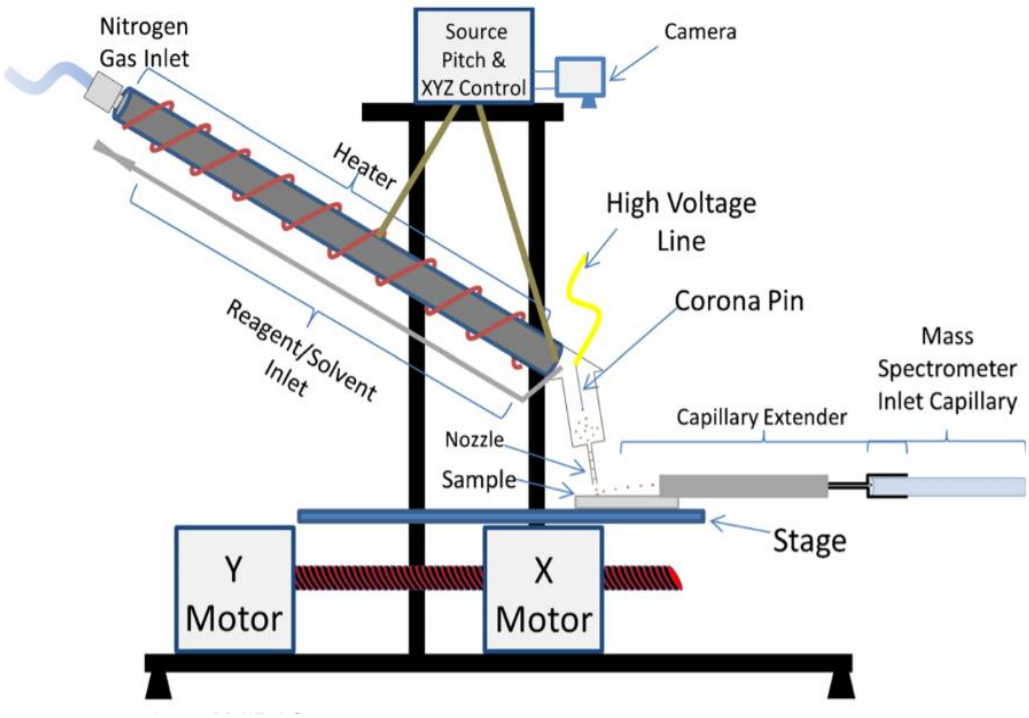


Figure 1.4. Schematic of Molecular Ionization Desorption Analysis Source (MIDAS) [33].

1.4.1. Ionization Mechanism

DAPCI sources, regardless of geometry and system parameters, are considered to be indirect sources, which means that the reagent ions are formed separately from the sample. Compounds in the sample follow Antoine's equation ($\log P = A - \frac{B}{T+C}$)

meaning that their vapor pressure (P) will increase with increasing temperature (T)[35-37]. Variables A, B, and C are component-specific constants, which suggest each analyte will respond differently[35-37]. When the temperature of the gas that creates the plasma increases, the desorption of a compound will increase, leading to its ionization. Heating the sheath gas can further improve the ionization efficiency and experimental sensitivity by liberating analytes from the surface of a sample in a thermal desorption type process[37-41].

Ionization Pathways, Conditions, and Characteristics

As with most mass spectrometry sources, ionization can occur in both positive and negative modes. Ion formation in DAPCI type experiments include those observed with APCI, such as protonation and deprotonation, adduct addition, and electron transfer. Properties of the analyte, including gas-phase basicity (GB), gas-phase acidity (GA), ionization energy (IE), and electron affinity (EA), determine the types of ions observed[33, 37]. Examples of the types of ions observed with DAPCI can be found in Table 1.3. In this research positive mode was the focus.

Table 1.3 Types of observed analyte ions

Polarity	**Observed Ions**
Positive	$[M+H]^+$, $M^{+\cdot}$, $[M+H_2O+H]^+$, $[M+NH_4]^+$, $[M+H-H_2O]^+$
Negative	$[M-H]^-$, $M^{-\cdot}$,$[M+Cl]^-$, $[M+O]^{-\cdot}$, $[M+OOH]^-$

Positive Ion Mode

In positive mode, ionization will take place as a proton transfer or from an interaction with a dopant. In the simplest case of proton transfer, atmospheric water can be used as the source of reagent ions[37, 42-45]. The positive reagent ion formation pathway is thought to follow an APCI like mechanism, originally proposed and studied in the 1970s, akin to that of a radioactive ^{63}Ni ion source[37, 42, 46-48].

$$N_2 + e^- \rightarrow N_2^+ + 2e^- \quad (1)$$

$$N_2^+ + 2N_2 \rightarrow N_4^+ + N_2 \quad (2)$$

$$N_4^+ + H_2O \rightarrow H_2O^+ + 2N_2 \quad (3)$$

$$H_2O^+ + H_2O \rightarrow H_3O^+ + {}^{\bullet}OH \quad (4)$$

$$H_3O^+ + H_2O + N_2 \rightarrow [(H_2O)_2 + H]^+ + N_2 \quad (5)$$

$$[(H_2O)_{n-1} + H]^+ + H_2O + N_2 \rightarrow [(H_2O)_n + H]^+ + N_2 \quad (6)$$

$$H^+(H_2O)_n + M \rightarrow M(H_2O)_{n-1}\,H^+ + H_2O \quad (7)$$

Ionization occurs when the water clusters impact the sample and transfer a proton to analyte molecules of higher proton affinity [37, 49]. The water clusters are formed by the nitrogen gas reacting with the electrons that are produced by the corona discharge creating the charged nitrogen (N_2^+) seen in reaction 1[33, 37, 47]. This then reacts further with additional nitrogen gas and water vapor (reactions 2-5) to eventually create the main ionization species, $H^+(H_2O)_n$ in reaction 6 and seen in Figure 1.6 [33, 37, 47]. When using a water cluster as the reagent, humidity influences ionization. Maximum signal intensity was found when the atmospheric relative humidity was

between 45-65%. Relative humidity levels above 75% promoted the dimerization of the analyte, while levels above 90% showed a pronounced decrease in signal[33, 37, 43]. Low humidity can cause a decrease in signal due to a limit amount of water clusters interacting with the sample. High humidity can also cause a decrease in signal because of the saturation of the plasma which can be extinguish.

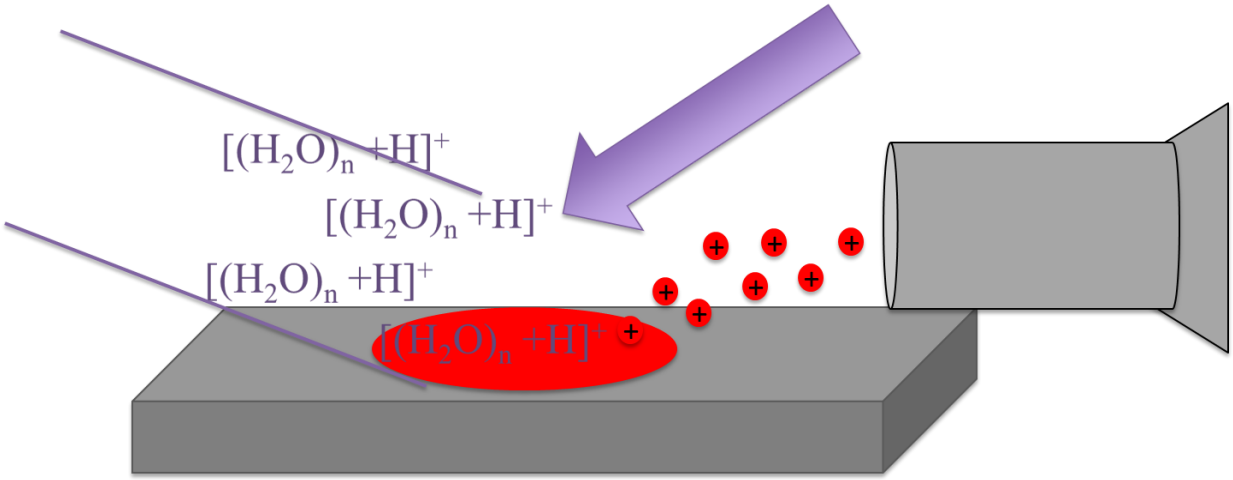


Figure 1.5. Schematic of the water cluster interaction with sample.

1.4.3. Time of Flight Mass Spectrometry

MIDAS is used in conjunction with a Time of Flight (TOF) mass spectrometer. Other mass spectrometers and detectors that have an atmospheric inlet can be used as well with the MIDAS. The TOF is a high resolution and sensitive detector and is ideal for quality control. When analyte ions enter the TOF from the MIDAS they are repelled by an electric field (labeled E in Figure 1.6) into the field free drift zone[50]. This zone is under vacuum pressure. When repelled by the electric field, the analytes separate by size in which the smaller analytes enter the drift zone first. There are two options to detect the analyte ions in the drift zone. The first is the liner detector that is

located at the end of the drift zone. The smallest masses will be detected first and the larger masses last. However, using this detector can increase the signal to noise ratio and can broaden the peaks[50]. The second option is to use the reflectron to reflect the analytes back towards the front of drift zone and into the reflectron detector. When the analytes hit the reflectron they are redirected by a positive potential towards the detector where all of the analytes will arrive at the detector at similar times[51]. The reason for this is that the smaller masses are traveling at higher potentials and will penetrate further into the reflectron compared to the larger masses[50]. Therefore, all the analytes arrive at the detector at similar times which improves the signal to noise ratio.

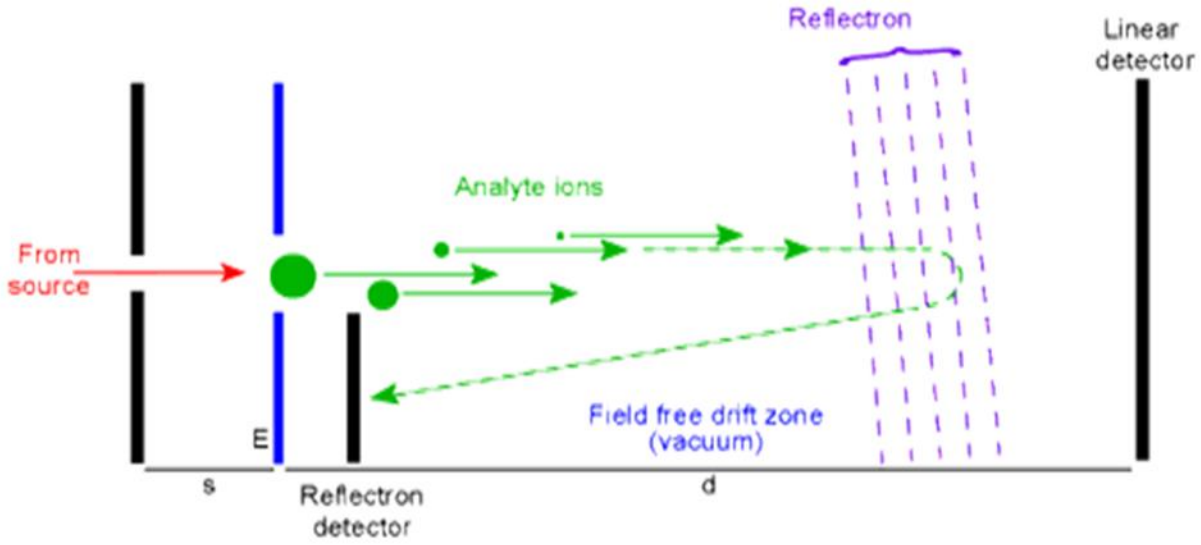


Figure 1.6. Schematic of time of flight mass spectrometry

1.4.4. MIDAS Sampling Plates and Applications

The most notable sampling platform used by the MIDAS is the Thin Layer Chromatography (TLC) plate holder. Developed TLC plates can be placed onto the

holder and compounds can be identified without the destruction of the plate. Winter *et al.* demonstrates the use of the TLC sampling platform by analyzing amino acids, analgesic tablets, and hop acids[34]. Figure 1.7 show the TLC plate holder and an image of a developed TLC plate being analyzed by the MIDAS. The plate sits between the nozzle and the capillary extender and is moved by the platform to analyze each spot separately. Using the TLC plate holder, Winter *et al.* were able to successfully detect caffeine, acetaminophen, acetylsalicylic acid, and salicylamide in an analgesic tablet, ionize arginine, leucine, lysine, methionine, and proline, and distinguish between the α- and β- acids in hop pellets[33].

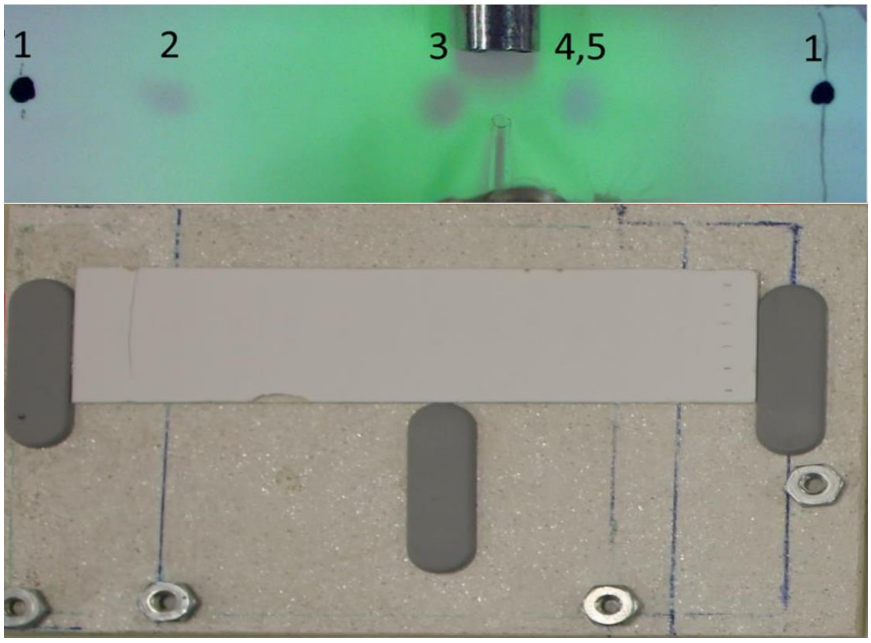


Figure 1.7. TLC plate holder for MIDAS analysis [33].

In conjunction with the TLC plate holder, Winter *et al.* explored other sample platforms[33]. Figure 1.8 displays the myriad of sample plates created for the MIDAS. The top two plates are 96 well plates that are covered with a foil to create shallow wells. The 96 well plates can be used to run 96 samples consecutively to

increase sample throughput. Using a smaller 96 well plates reduces the amount of volume needed and can be ideal for “precious” sample analysis. Figure 1.8C is a stainless steel 384 Matrix Assisted Laser Desorption Ionization (MALDI) plate and it is used to further increase sample throughout. The caveat to this plate is that samples must be solid or dry before analysis. On the right of the MALDI plate is the “pill” holder that is designed to hold pharmaceutical tablets or larger solids in place for surface analysis. The last sampling plate acts likes a vacuum. When analyzing samples that are easily moved by the nitrogen gas flow, the vacuum plate can be used to hold the sample in place. This can be used for paper and cloth samples.

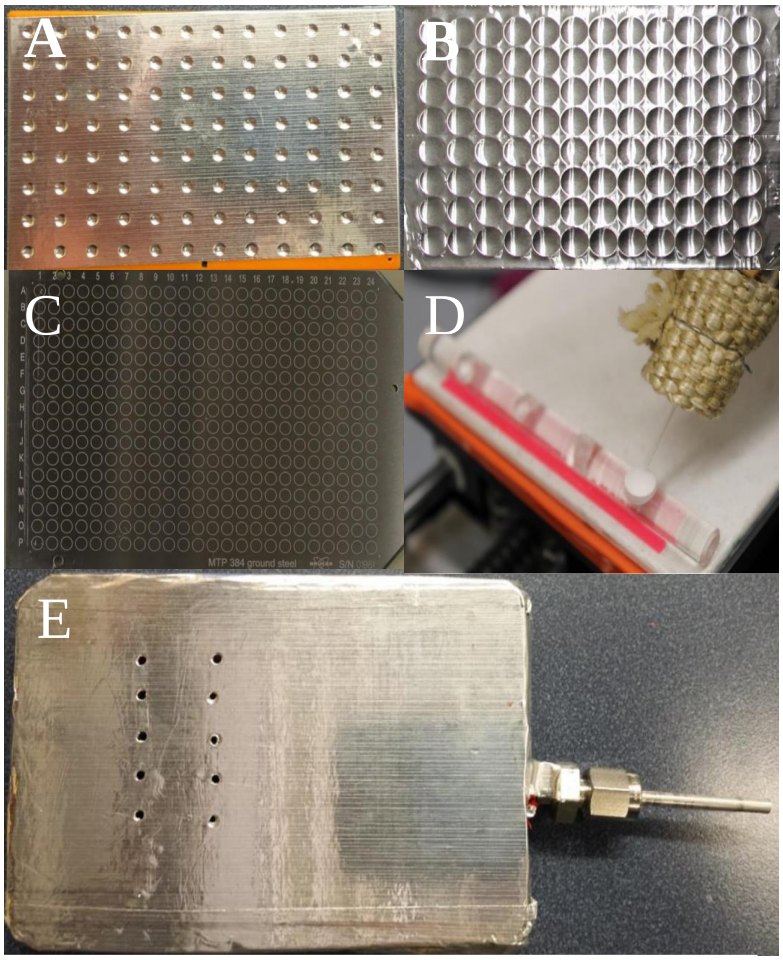


Figure 1.8. MIDAS sampling plates. A) shallow 96-well plate B) Custom 96 well-plate C) MALDI plate D) pill holder E) vacuum plate

1.5. Research Overview

With the interchangable sampling platforms, the MIDAS has the capability of being a powerful tool for rapid food authenticity and adulteration detection. The goal of this research is to display the versatility of MIDAS as an analytical tool for several applications with the focus being on food adulteration detection and authenticity. MIDAS was utilized to characterize and assay vanilla extracts, vanilla beans, and black peppercorns. The analysis of vanilla beans and black peppercorns spurred the creation of additional sample platforms to secure solid samples under the gas stream during analysis. Vanilla extracts, beans, and peppercorns display the versatilty of the MIDAS to be used for liquids, pastes, and solids.

In addition to food analysis, the MIDAS has the capability of being utlized in the forensic field. Rapid detection techniques such as chromatographic and other ambient ionization sources that have been used in the food field overlap those seen in the forensic field. An exploration project of anlyzing metabolites of THC and nicotine in synthetic earwax was conducted to fruther diaplsay the versatilty of the MIDAS as an ionization source. Cotton swabs were introduced for earwax analysis and a new diagnostic technique was proposed. Using swabs and synthetic ear wax, nicotine and cannabis metabolites were explored. Earwax is neither a solid nor a liquid and therefore further displays the ability of the MIDAS to be used for all sample types. MIDAS is a rapid, powerful, and flexible anlaytical tool that can be applied to serveral sample types (i.e. powders, pasters, solids, waxes) and therefore would be advatageous to many fields of study.

Chapter 2: Materials and Methods

2.1. Instrumentation

2.1.2. MIDAS Parameters

MIDAS was used as the ionization source for all experiments. Nitrogen gas pressure was set at 80 pounds per square inch. Incident angle of the nozzle was set at 50º. Nozzle to sample distance and source to inlet distance was 2.5mm and 2mm, respectively. Linear velocity, current, and voltage were 3.6 m/s, 12µA, and 3.0kV, respectively. Previous MIDAS work determined that the facility that the MIDAS is housed in has a relative humidity range between 18% and 50%[33].

2.1.3. MIDAS Optimization

Original work with the MIDAS included a glass nozzle attached to the end of the source (Figure 2.1) to direct the gas stream to a small spot on a TLC plate. The glass nozzle was beneficial for small sample areas but was not ideal for larger samples such as vanilla beans or liquid samples on the 96-well plate. The glass nozzle was designed for small areas and therefore when used with some of the samples, the samples would show signs of degradation (browning or burning). Removing the nozzle increased the surface area to distribute the heat. Removing the nozzle also eliminated the risk of repairing the glass or contaminating the nozzle with the sample.

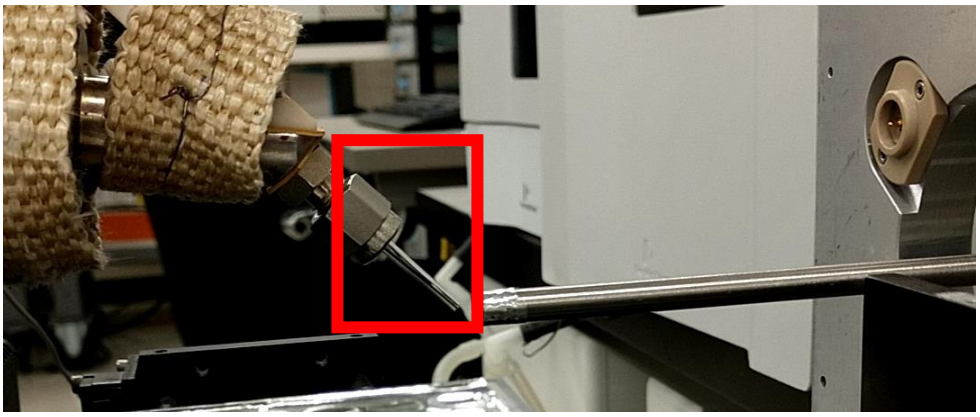


Figure 2.1. MIDAS glass nozzle and capillary extender.

With the removal of the glass nozzle the optimal gas pressure was assessed. With the nozzle, the pressure was set at 40psi with linear velocity of 3.0 m/s. Removing the nozzle, the pressure had to be adjusted to keep the same operating linear velocity. Gas pressures ranging from 20 to 120psi were tested to determine the optimal nitrogen flow. As the pressure increased the signal intensity increased. Pressures at 80psi delivered linear velocities around 3.0 m/s, which was comparable to previously published methods for the MIDAS. Pressures the ranged above 80psi inhibited sampling performance by blowing samples off the plate before proper ionization occurred.

Optimal distances between the nozzle and the sample were reevaluated to ensure that 2.5mm was still the optimal distance. The 96-custom well plate was the targeted plate for analysis and was used to test the optimal distances. Caffeine (195.0 $[M+H]^{+)}$ is a well-known standard and was used in conjunction with a red Sharpie to test signal intensity. Red Sharpie contains a compound called rhodamine red (568.0 $[M+H]^{+}$) and has been used alongside caffeine in original MIDAS optimization. Caffeine and

rhodamine were spotted on alternating spots across the 96-well plate. Twelve sample spots in total spanned across the top, middle, and bottom of the plate to assess the uniform ionization of the plate. Nozzle (source nozzle) to sample distance and nozzle to inlet (capillary extender) distance was tested from 0.5mm to 4mm in 0.5mm increments. Maximum signal intensity was found at 2.5mm and 2mm for nozzle to sample and nozzle to inlet, respectively. All samples were assayed at these fixed distances. The MIDAS sits on an adjustable table and the height of the platform can be adjusted to these parameters.

Caffeine, rhodamine, and APCI tune mix were used for all optimizations. The use of these compounds aid in testing the efficacy of each new sampling platform explored throughout this research. Each platform was tested in a similar way to insure uniform ionization across the plates.

2.1.5. TOF Software and Parameters

Perkin Elmer AxIon and AxIon2 Time-of-Flight mass spectrometer with TOF MS Driver software version 4.2 (Waltham, MA, USA) were used to analyze all the samples. All samples were acquired in positive mode at 1.0 spectra$\cdot s^{-1}$ [34]. Voltages for the cylinder, endplate, and capillary entrance were 0V[34]. The capillary exit, skimmer, radio frequency (RF) voltage, and offset voltage were 175, 18, 470, and 12V, respectively for the AxIon. The capillary exit, skimmer, radio frequency (RF) voltage, and offset voltage were 80, 25, 500, and 10.7V respectively for the AxIon2.

2.1.6. TOF Tune

Agilent APCI/APPI tuning mix (Santa Clara, CA, USA) was used to tune the mass spectrometer before the analysis of all the samples. Tune mix is spotted on a sampling plate and ran at corresponding experimental temperatures. Running tune mix at the same temperature as the samples will ensure that the instrument is ionizing correctly at that set temperatures. Tune mix is spotted (10µL) onto a glass plate and placed under the gas stream for ionization. Figure 2.2 displays the Agilent provided expected peaks associated with the tune mix.

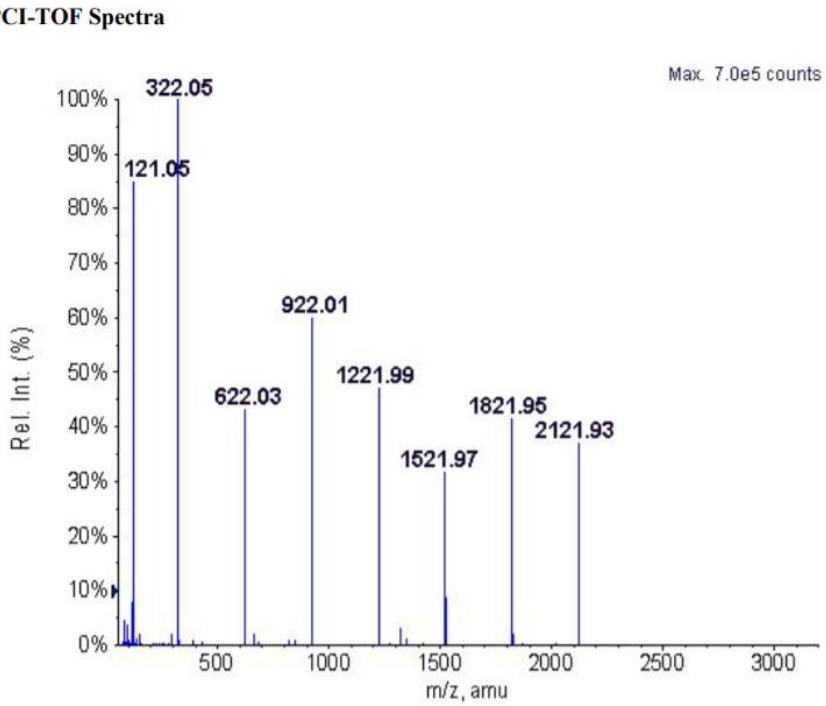
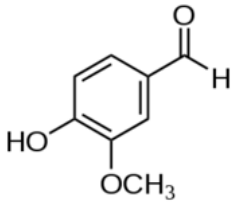


Figure 2.2. Expected spectra from APCI tune mix from Agilent.

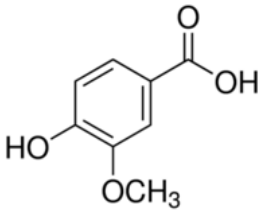
2.2. Materials

2.2.1. Vanilla Characterization

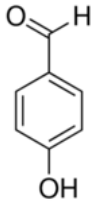
Vanillin, vanillic acid, vanillyl alcohol, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, 4-hydroxybenzyl alcohol, 3,4-dihydroxybenzoic acid, ethyl vanillin, piperonal, 3,4-dihydroxybenzaldyhe, and coumarin standards were purchased from Millipore Sigma (Burlington, MA, USA).



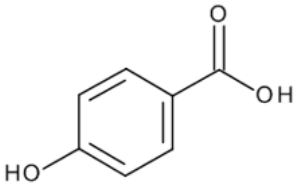
Vanillin



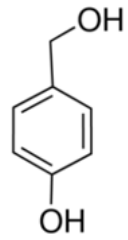
Vanillic Acid



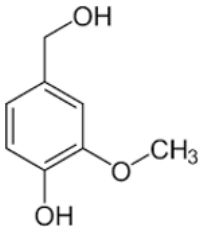
4-Hydroxybenzaldehyde



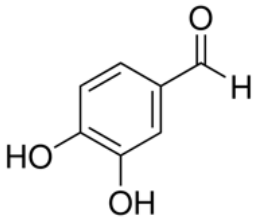
4-Hydroxybenzoic Acid



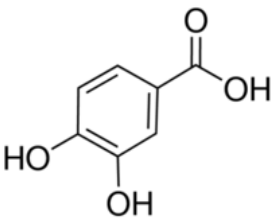
4-Hydroxybenzyl Alcohol



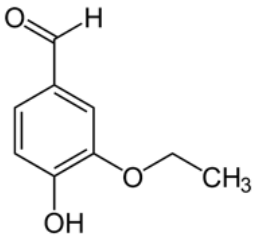
Vanillyl Alcohol



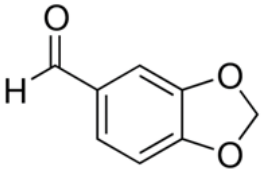
3,4-Dihydroxybenzaldehyde



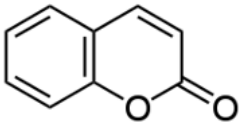
3,4-Dihydroxybenzoic Acid



Ethyl Vanillin



Piperonal



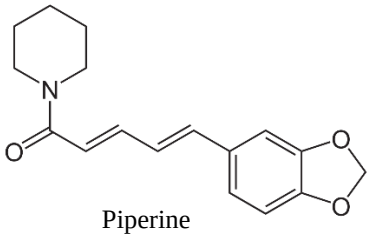
Coumarin

Ethanol (200 proof) was purchased from Pharmco (Greenfield Global, Brookfield, CT, USA). Pure vanilla extracts and imitation vanilla extracts were purchased from local grocery stores (pure vanilla 1-3 and imitation vanilla 1-3). Indian, Indonesian,

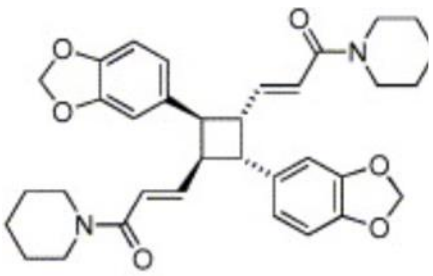
Madagascan, Sri Lankan, Tahitian, and Tanzanian beans were provided by McCormick & Company. Mexican and Tonka beans were purchased Edson ca Inc (Fresh Bean Company, Sanford, FL, USA) and L'epicure du Chef (Fondettes, France) respectively. High purity water and conical tubes were purchased from VWR International (Radnor, PA, USA). Double sided tape purchased from A&J suppliers and glass slides were used for solid bean analysis. Custom made large 96-well plate was used for the liquid samples.

2.2.2. Black Peppercorn Analysis

Piperine was purchased from Millipore Sigma (Burlington, MA, USA). Ethanol (200 proof) was purchased from Pharmco (Greenfield Global, Brookfield, CT, USA).



Piperine



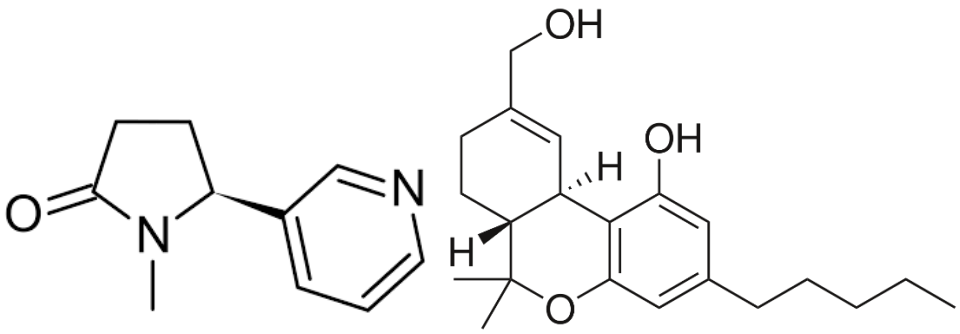
Dipiperamide

Black peppercorns were provided by McCormick & Company. Papaya seeds were extracted from a Maradol papaya purchased from the local grocery store. Double sided tape purchased from A&J suppliers (Cardiff, England). Olde Thompson 4-1/2 Monterey Peppermill purchased from Old Thompson Inc. (Oxnard, CA, USA). Conical tubes purchased from VWR International (Radnor, PA, USA). Glass slides.

Figure 2.3. Papaya seeds within the fruit of the papaya

2.2.3. Exploration of Forensic Compounds

Cotinine and 11-Hydroxy-Δ^9-tetrahydrocannabinol were purchased from Millipore Sigma (Burlington, MA, USA).



Cotinine 11-Hydroxy-Δ^9-tetrahydrocannabinol

Ethanol (200 proof) was purchased from Pharmco (Greenfield Global, Brookfield, CT, USA). Synthetic ear wax purchased from Biochemazone (Edmonton, Alberta, Canada). Sterile cotton swabs were purchased from Puritan (Guilford, ME, USA). Conical tubes were purchased from VWR International (Radnor, PA, USA).

2.3. Methods

2.3.1. Custom 96-well Plate Reproducibility

The custom 96-well plate was used for the vanilla extract analysis and therefore the spot-to-spot reproducibility was tested across the plate (Figure 2.4). Samples were spotted (10µL) on a 96-well plate with a pipette starting at spot A2 and then every other spot after that. Spot reproducibility was high across the plate and a similar trend was seen down the plate (A2-G2) and has an RSD of 11%. Therefore, the entire plate can be utilized for vanilla extract analysis.

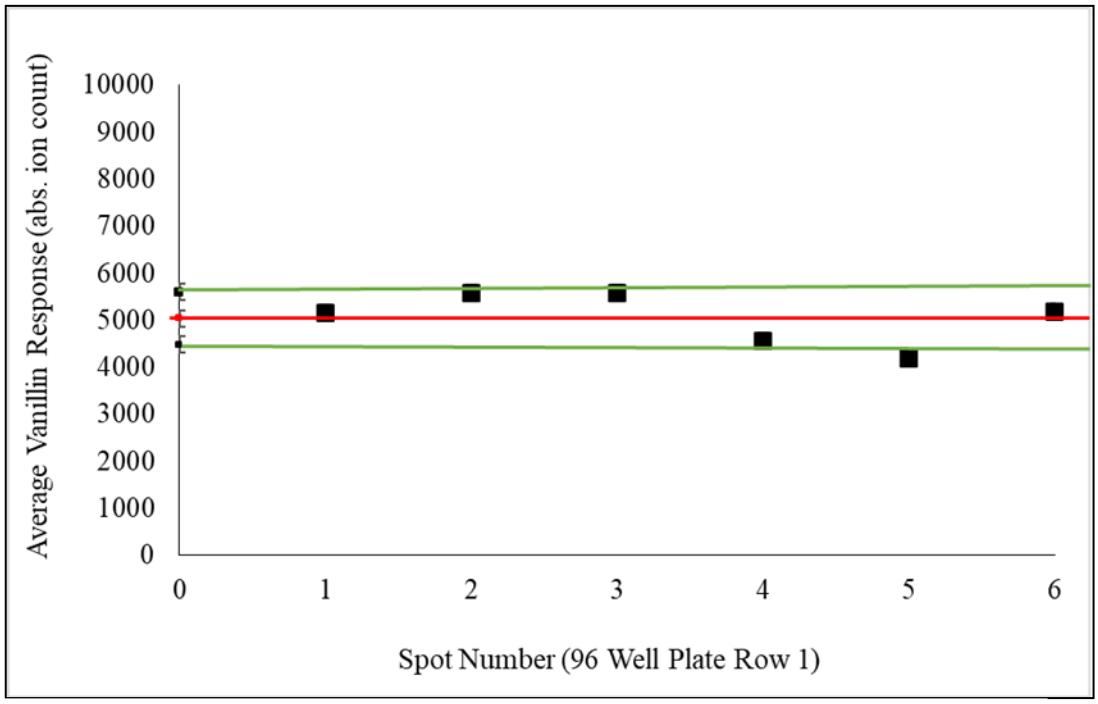


Figure 2.4. Reproducibility of the custom 96-well plate using the 1mg/mL vanillin standard.

2.3.2. Vanillin and Ethyl Vanillin Temperature Mapping

Vanillin and Ethyl Vanillin standards (1mg/mL) were spotted on a 96 well plate and were analyzed immediately. On a custom 96-well plate the first column was left blank, and the first sample was spotted in column two. Each sample (10µL) spotted

on the plate contained a blank spot next to, above, and under it to prevent sample migration underneath the gas stream and potential contamination of the neighboring sample spots (Figure 2.5).

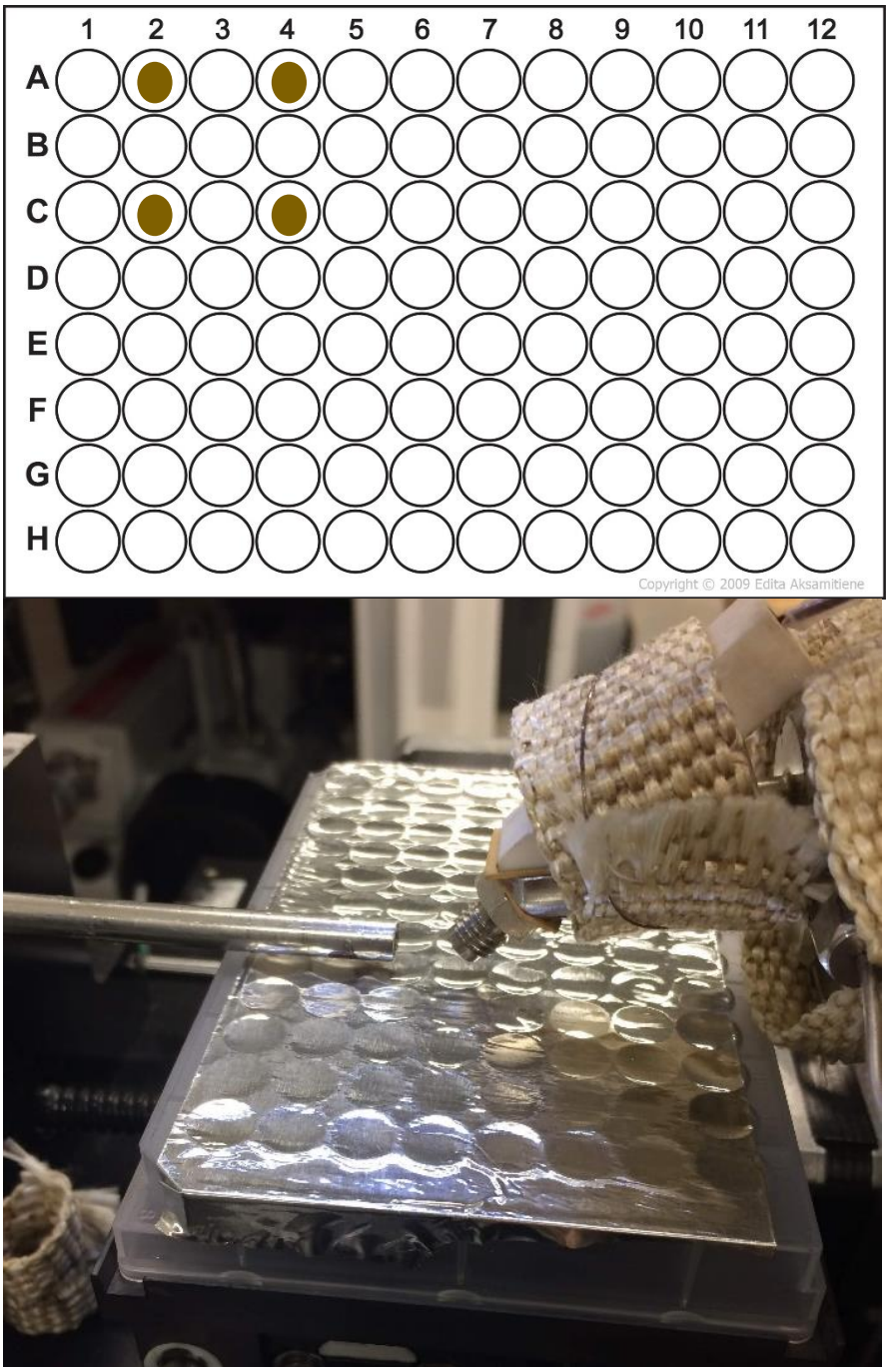


Figure 2.5. Schematic of the spotting used on the custom 96-well plate.

The spots were assayed at temperatures ranging from 25º to 185ºC (measure by a K-type thermocouple). The thermocouple was held in a fixed position using a metal alligator clip stand. The thermocouple was placed in between the gas nozzle and the mass spectrometer inlet extension to read the gas stream temperature (Figure 2.6). The temperature was controlled by a variable power supply attached to the heating coils of the MIDAS' body. The optimized temperature for both compounds occur around 100ºC and therefore all vanilla samples were analyzed at that temperature.

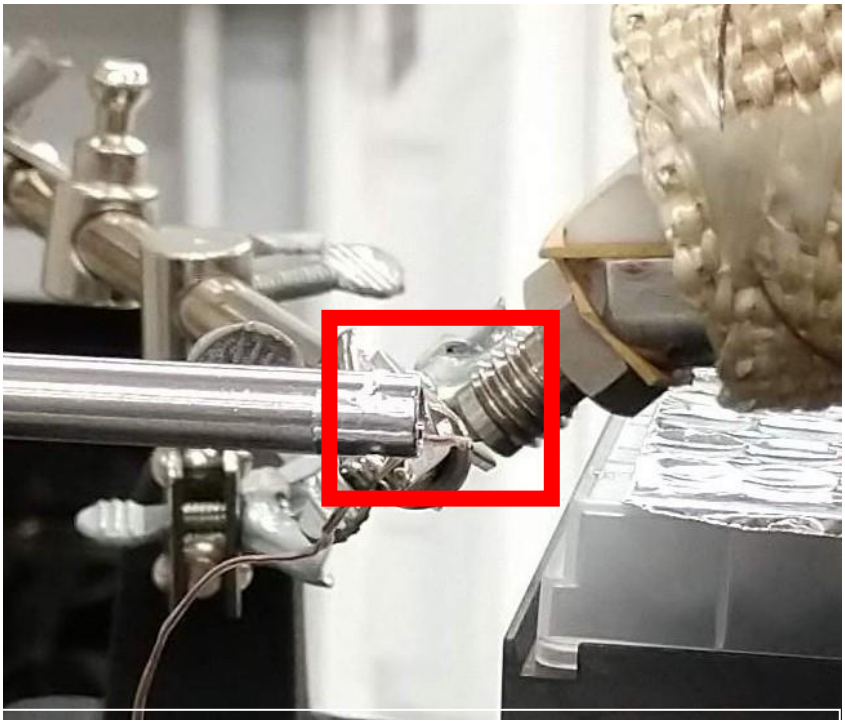


Figure 2.6. MIDAS configuration with thermocouple placed in between nozzle and capillary extender.

2.3.3. MIDAS Vanilla Characterization

Vanilla Extract Analysis

Vanilla standard solutions were diluted in 200 proof ethanol at a concentration of 1000ppm for rapid spot analysis. The standards were spotted in triplicate on the 96 well plate as described in the previous section. Base peaks (the peak with the highest

intensity) were determined for each of the vanilla standards in positive ionization mode and were used as identifiers throughout the vanilla extract analysis. Bean extracts were made by soaking 20mg of vanilla bean pieces in 1mL of 200 proof ethanol for 5 days. One of the methods of making vanilla extract is to have an ethanol/water mixture circulate over the beans for about 2-3 days. Another method consists of “pulverizing” whole beans and circulating ethanol over the bean for 8-9 days. Since the beans were neither whole nor pulverized, a method of soaking 20 mg of shredded beans in a 35/65 ethanol/ water mixture for 5 days was determined. The extracts were placed in 15mL conical tubes and stored in the dark at room temperature during the extraction process (Figure 2.7). The ethanol/water mixture was determined by previous literature methods as well as the mixture is commonly used in commercial vanilla extracts.

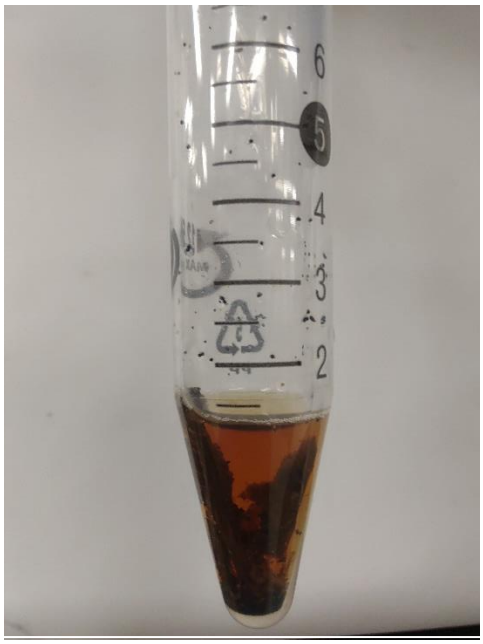


Figure 2.7. Vanilla bean extraction in conical tube.

Testing adulteration of vanilla extracts, coumarin was spiked into a randomly chosen pure vanilla extract that did not contain coumarin prior to spiking from 0.01% to 0.75%(v/v). A curve was constructed by averaging the spectral intensities of the vanilla and coumarin peak for the spiked sample and plotting the ratios. In addition to exploring adulteration responses, a calibration curve was constructed to determine the concentration of vanillin in each of the extracts. The calibration curve was constructed by diluting a 1000ppm vanillin standard (35:65 ethanol to water) to create a curve range from 10 to 150ppm and analyzed in triplicate.

Vanilla Bean Analysis

Vanilla bean pieces (mixture of whole and shredded beans) were placed on an adhesive plate and analyzed directly. The adhesive plate was constructed by placing double sided tape on a glass plate in organized columns. The first column contained one single adhesive strip that served as a blank (B) for background subtraction. The following six columns contained three adhesives strips that were assigned to each of the countries (Figure 2.8). The beans that were provided by McCormick and Company (Indian (I), Indonesian (In), Madagascan (M), Sri Lankan (S), Tahitian (T), and Tanzania (Tn)) were assayed together using the plate set up in Figure 2.8. The Mexican and Tonka beans were purchased separately and therefore were assayed on separate plates. Tonka beans were separated from the Mexican beans to prevent potential contamination.

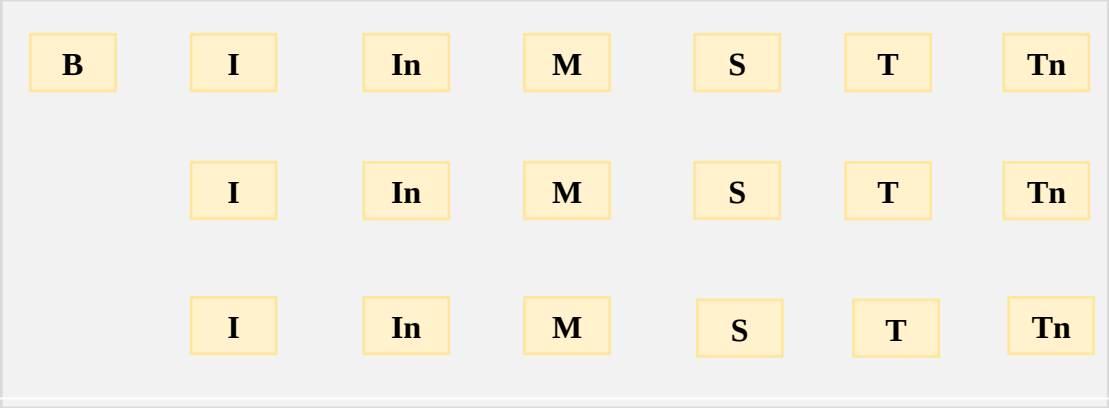


Figure 2.8. Layout of country-of-origin vanilla bean analysis using adhesive sampling plate.

2.3.4. HPLC Vanilla Extract Characterization

A PerkinElmer Flexar UHPLC system was used as a complementary technique to the MIDAS analysis. A method was created to determine the concentration of vanillin in the vanilla extracts. Reproducibility of the system was tested by running caffeine and 50ppm vanillin in triplicate on the system. A standard curve was created using the method stated in Table 2.1 to determine the linearity range, limit of detection, and limit of quantification. The range was determined to be 5-100ppm. All extracts were tested against the curve to determine the vanillin content using a bracketed quantification technique.

Table 2.1. Parameters used for HPLC characterization

Column	Phenomenex Luna 5µ C18 Particle Size/type: 5 µm octadecylsilyl (ODS) groups Dimension: 250 x 4.6mm
Mobile Phase	Solvent A: Water w/ 0.1% Formic Acid Solvent B: Acetonitrile (ACN) with Solvent Program: isocratic 65% A/ 35% B
Flow Rate	1.0 mL/min
Oven Temperature	30 C
Detection	PDA wavelength 280
Injection Volume	50uL
Sampling Rate	20 points/second
Run time	10 minutes

2.3.5. Piperine Temperature Mapping

Piperine is the main compound in black peppercorns and therefore was used as the standard for temperature optimization. A 1000ppm standard solution was made by dissolving 1mg of piperine in 1mL of 200 proof ethanol. A cotton swab was then dipped into the solution and placed underneath the gas stream at temperatures ranging from 25 ºC to 190 ºC (max setting). The swab was used to imitate a peppercorn surface. The optimal temperature was determined to be 125ºC and was used for further pepper characterization.

2.3.6. Piperine Quantitation

The piperine standard that was used for temperature mapping was tested in triplicate to identify the base peak. This base peak was used an identifier in all pepper samples. A piperine calibration curve with concentrations ranging from 1ppm to 10ppm was constructed to explore piperine quantitation. The curve was made by diluting the 1000ppm standard in ethanol.

2.3.7. Whole and Ground Black Peppercorn

Whole black peppercorns (Figure 2.9) from McCormick & Company and papaya seeds were used for characterization. Papaya seeds are the most common adulterant of black pepper and therefore were used for characterization. The papaya seeds were extracted from a fresh papaya that was purchased from a local grocery store. The seeds were washed and left to sit out and dry for 24 hours. Both peppercorns and the papaya seeds were placed on the 96-well plate and an adhesive holder was placed on top to prevent moving during analysis.

Figure 2.9. Black peppercorns provided from McCormick from different origins.

In addition to whole corns, ground peppercorns were assayed. Black peppercorns were placed into a pepper grinder to be ground and placed on an adhesive plate. The pepper grinder was tightened all the way (to not allow movement) and then was loosened by a half turn to grind the pepper and papaya samples. The grinder was thoroughly cleaned after each use. The same method for the vanilla bean analysis was used for the ground peppercorn and papaya samples (Figure 2.8).

Five blind samples for both whole and ground peppercorn were analyzed to determine the presence or absence of papaya seeds. The blinds consisted of a mix of four peppercorns or papaya seeds.

2.3.7. Ear Wax Samples

Metabolites of nicotine and cannabis in ear wax were explored. Cotinine and 11-Hydroxy-Δ^9-tetrahydrocannabinol were assayed separately. Both standards were

tested using the same method as the piperine standard where a cotton swab was dipped into the solution and placed under the gas stream. The cotton swab was used because of the frequent use of swabs for ear wax analysis. The optimal temperature for cotinine and 11-Hydroxy-Δ^9-tetrahydrocannabinol was determined to be 150°C. Calibration curves for each compound were constructed to determine the limit of detection and limit of linearity. Synthetic ear wax was used as a consistent matrix for the analysis of the two metabolic compounds. Concentrations of the metabolites (10-750ppm) were analyzed in the synthetic ear wax matrix and compared to those in the ethanol matrix.

Chapter 3: Rapid Characterization of Vanilla

This chapter is reproduced in part from: ***Pitman, C.N.*** *and W.R. LaCourse, Rapid Characterization of Vanilla with Molecular Ionization Desoprtion Analysis Source (MIDAS) for Mass Spectrometry. International Journal of Mass spectrometry. Under Review.*

3.1. Introduction

Vanilla is one of the most common flavorings used in food and beverage products[7-13]. With consumer tastes shifting toward all-natural products, the costliness of harvesting of vanilla, and a range of other market forces, vanilla extract has become a target of adulteration[8, 11-17]. Commonly, enhancers such as ethyl vanillin and coumarin are added to extracts to create a cheaper product. The United States FDA has banned coumarin, which is used to enhance flavor in food products, due to its hepatotoxicity[1, 52]. However, it is still allowed in small quantities in other countries such as Mexico and the European Union[7, 9, 12, 16, 18, 19] . Other countries have a limit of consuming 0.1 mg/kg of body weight of coumarin per day[53]. The European Parliament and of the Council state the maximum level of coumarin in desserts to be 5 mg/kg[54, 55]. Therefore, techniques for quality control are needed to screen for the presence of potential toxins and other breaches of authenticity. A screening process is also needed for identifying country of origin. If the origin of the beans/extract is not correctly disclosed, it can cause a consumer to make a misinformed purchase. Some vanillas beans are purchased for specific uses, such as baking, and therefore if the incorrect product is purchased it can cause an undesired product and potential loss of money[56]. This chapter will discuss how MIDAS is used as a rapid characterization method for vanilla extracts and beans. The chapter will also demonstrate the ability of the MIDAS to be used a fast identification

technique for compounds in liquid and plant matrixes. As well as the discovery of two new sampling platforms for MIDAS analysis.

3.2. Results and Discussion

MIDAS platforms have been constructed, but not all that were listed in chapter 1 were utilized. The custom 96-well plate allows for liquids to be assayed rapidly. The shallow wells contain the liquid within one spot and prevents sample movement during analysis. Vanilla extracts were used to highlight the ability of the 96-well plate to be an effective sampling platform for liquids.

3.2.1. Vanillin and Ethyl Vanillin Temperature Mapping

Prior MIDAS analysis has been conducted at 185 ºC and therefore was used as the starting temperature for vanilla analysis. Analysis of vanilla standards and vanilla extracts suggested that the temperature of 185 ºC caused a decrease in ionization and resulted in degradation of the sample. Vanilla extract samples appeared "burnt" and degraded upon impact of the nitrogen stream. The degradation prompted exploration of alternative ionization temperatures.

Vanillin is the main compound associated with vanilla and usually the most abundant compound in extracts and beans. Ethyl vanillin is the most common additive in vanilla extracts due to its pungent scent and taste which makes it is commonly listed as an ingredient for imitation vanilla extracts. The two compounds were used to test ionization temperatures ranging from 25 ºC (ambient temperature) to 185 ºC (highest temperature possible) and the intensities were plotted. Figure 3.1 represents the

temperature vs mass intensity plot of vanilla and ethyl vanillin. The plot suggests that the optimal ionization temperature falls around 100 ºC. Shown by the error bars, there is signal fluctuation seen at 100 ºC which could be indicative of saturation of sample signal. The temperature mapping occurred at a concentration of 1000ppm which is much greater than the 150ppm limit of linearity that was determined later in this chapter. To explore the fluctuation further, a lower concentration such as 100ppm could be utilized. However, using a lower concentration may not be ideal when doing temperature mapping because there is a risk of no or significantly decreased signal at non optimal temperatures. At 100ppm the temperature mapping curve would yield the same outcome with less signal fluctuation at 100 ºC.

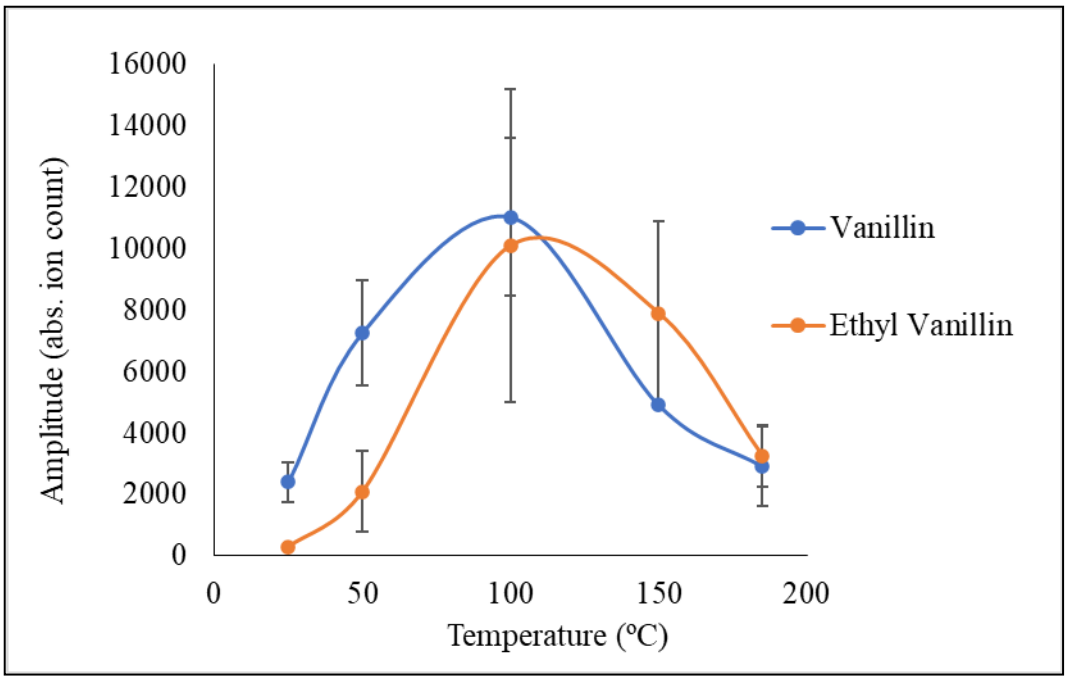


Figure 3.1. Temperature map of 1mg/mL vanillin and ethyl vanillin standards.

Literature suggests that temperature plays a role in choosing between pure and imitation vanilla extracts for baking. If internal temperatures, of baked goods, reach above 135 ºC pure vanilla extracts will evaporate and the “vanilla taste” will not

retain[57]. Therefore, lower temperatures are more ideal when assaying pure vanilla extracts. Based on the temperature mapping outcome and the literature results, it was determined that 100 ºC was the ideal temperature to carry out vanilla characterization. Since all standards and extracts were originally tested at 185ºC, the process was repeated at 100ºC (the optimal temperature) and a significant increase in signal was observed. Figure 3.2 shows an example of the signal differences between 185ºC and 100ºC. All vanilla compounds including vanillin (153.1 $[M+H]^+$) increased in signal at 100ºC.

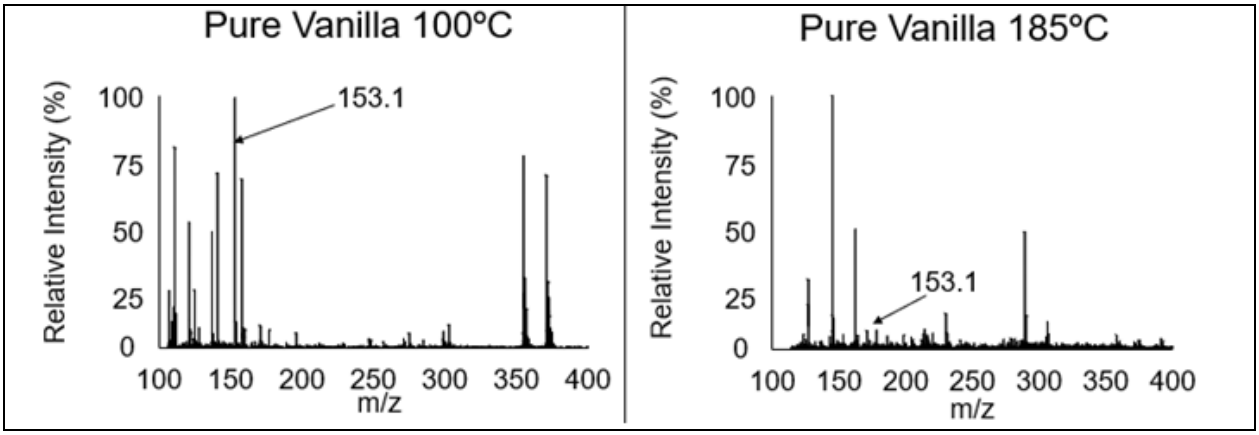


Figure 3.2. Vanilla extract comparison at 100°C and 185°C. Vanillin (153.1 $[M+H]^+$) intensity increased at 100ºC.

3.2.2. Vanilla Standards

Vanilla bean extracts contain a variety of compounds, with the most common being vanillin (Figure 3.3(A))[8, 9, 11, 12, 15, 56, 58-60]. Table 1 displays the compounds found in pure vanilla extracts (a-d) and common additives (e&f) [7-9, 11, 12, 16, 19, 59].Vanilla standards were assayed, and the base peaks (m/z) were recorded in Table 3.1. The base peaks were used to identify vanilla compounds in the vanilla extracts. Figure 3.3 displays the vanillin (A) and ethyl vanillin (B) standards. Using positive

ionization mode, a gain of a proton is expected for most compounds. Both vanillin and ethyl vanillin gain a proton when ionized.

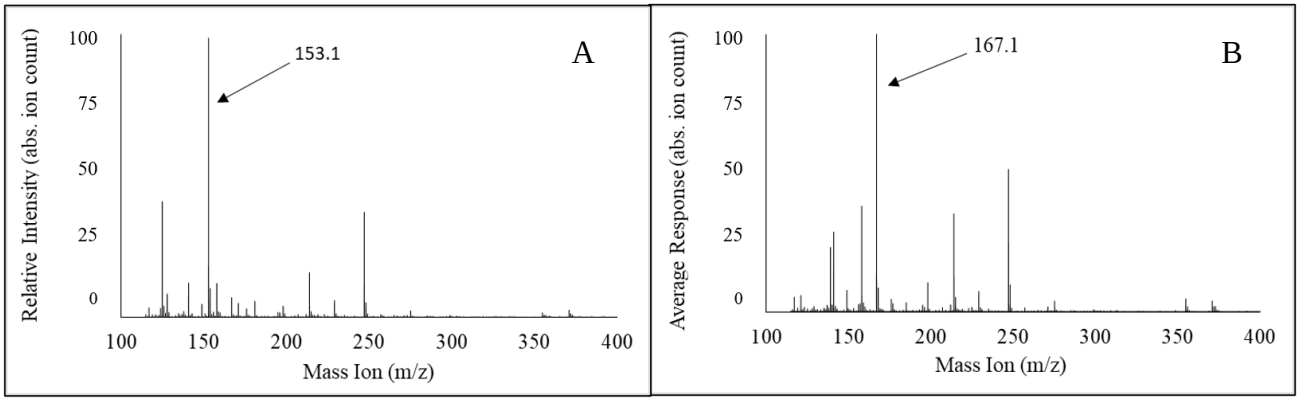


Figure 3.3. A) Vanillin (153.1) and B) ethyl vanillin (167) standards at 100°C. Both compounds display a gain in proton.

In Table 3.1, most of the compounds gained a proton ($[M+H]^+$) except for three. Vanillyl alcohol, 4-hydroxybenzyl alcohol, and 4-Hydroxybenzoic acid displayed base peaks that demonstrate a loss of a hydroxyl group (-OH). Hydroxyl loss in positive mode can occur due to the interaction of water clusters in the desorption/ionization process[61]. The loss of a hydroxyl is commonly seen with acids in positive mode. The water cluster's interaction with one of the hydroxyl groups can create a leaving group and therefore will result in a loss of a hydroxyl group ($[M-OH]^+$). Negative mode ionization could alleviate this problem but is not suitable for all compounds. When testing in negative mode, not all compounds were detected and therefore all analysis was carried out in just positive mode. Further exploration of mode switching could be explored but was not the intent of this project.

Table 3.1. Compounds commonly found in pure and imitation vanilla extracts. The last three compounds are the most common additives.

	Compound	Molar Mass (g/mol)	Base Peak (m/z)
a	4-Hydroxybenzyl alcohol	124.1	125.1 $[M+H]^+$
b	4-Hydroxybenzoic acid	138.1	121.0 $[M-OH]^+$
c	Vanillin	152.2	153.1 $[M+H]^+$
d	Vanillyl alcohol	154.2	137.1 $[M-OH]^+$
e	Coumarin	146.1	147.0 $[M+H]^+$
f	Ethyl vanillin	166.2	167.1 $[M+H]^+$

3.2.3. Vanilla Extracts

Pure and imitation extracts were purchased from local stores to assay and characterize. The intent was to identify vanilla compounds in both pure and imitation extracts. Purchased extracts were assayed using the MIDAS-TOF-MS. Figure 3.4 displays the six total extracts that were assayed. The pure vanilla extracts (Figure 3.4 A-C) do not contain ethyl vanilla which is a positive indication that the extract is authentic. Another indication of pure vanilla extract is the complexity of the spectra that is seen in the pure vanilla and not in the imitation vanilla. This is expected due to the complexity of plant-based samples.

The imitation vanilla extracts (Figure 1 D-F) contain ethyl vanillin which is a confirmation that the extract is not pure and that the ingredients stated on the bottle are accurate. Pure vanilla compounds were detected in the imitation extract which may be an indication that a pure vanilla extract was used as a base for the imitation vanilla, but further analysis is needed to conclude that result. The additive of concern, coumarin, was not detected in any of the extracts. This compound is banned in the United States and therefore should not be in any of the extracts that were purchased.

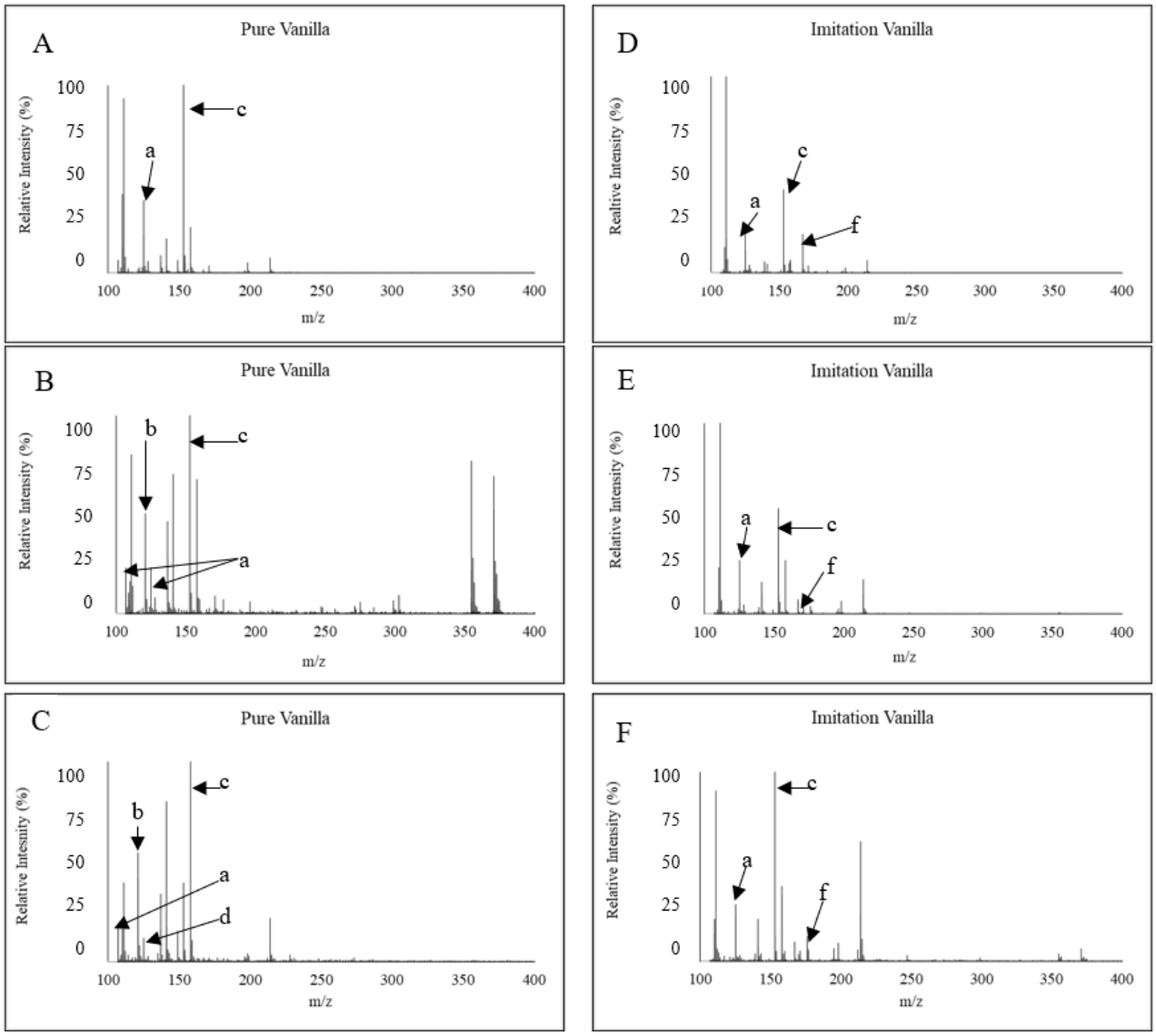


Figure 3.4. Pure (A-C) and imitation (D-F) vanilla extracts analyzed at 100ºC. The pure vanilla extracts all contain the expected vanillin peak (A, $[M+H]^+$=153.1). Similarly, in all the imitation vanilla extracts the ethyl vanillin peak (I, $[M+H]^+$=167.0) was detected. In addition to vanillin and ethyl vanillin, other commonly found vanilla compounds were characterized. The following were characterized;4-hydroxybenzyl alcohol (E, $[M+H]^+$=125.1, [M-OH]=107.1), 4-hydroxybenzoic acid (D, [M-OH]=121.0), vanillyl alcohol (F, [M-OH]=137.1).

Table 3.2 displays all the detected compounds in the vanilla extracts. Individually purchased pure vanilla 1, 2, and 3 are indicated in the table as 1,2,3, respectively under the pure vanilla column. Similarly, the three imitation vanillas purchased are

labeled 1,2,3 under the imitation column. In addition to vanillin and ethyl vanillin, other major vanilla compounds such as vanillyl alcohol (137.1 $[M\text{-}OH]^+$), 4-hydroxybenzyl alcohol (125.1 $[M+H]^+$, 107.1 $[M\text{-}OH]^+$), and 4-hydroxybenzoic acid (121.0 $[M\text{-}OH]^+$) were detected, confirming authenticity in the pure extracts. Not all compounds listed in Table 3.1 were present in every extract, which is expected as not all extracts will contain every component. The difference in pure vanilla 1 compared to other two extracts may be the indication of its origin or the type of vanilla bean used. This is explained further in section 3.5.

Table 3.2. Vanilla compounds detected in three different pure vanillas and three different imitation vanilla extracts.

Compound Detected	**Individual Pure Vanilla**			**Individual Imitation Vanilla**		
	1	**2**	**3**	**1**	**2**	**3**
4-hydroxybenzoic acid; $[M\text{-}OH]^+$=121.0		●	●			
4-hydroxybenzyl alcohol; $[M+H]^+$=125.1	●	●	●	●	●	●
vanillyl alcohol; $[M\text{-}OH]^+$=137.1		●	●			
vanillin; $[M+H]^+$=153.1	●	●	●	●	●	●
ethyl vanillin; $[M+H]^+$=167.0				●	●	●

Identifying vanilla compounds in the extracts highlighted the ability of the MIDAS to be used a rapid qualitative technique. Varying intensities in the pure vanilla extracts led to the exploration of testing the MIDAS's ability to quantify vanillin. Figure 3.5 (C) displays the calibration curve constructed with a linearity range of 10-150ppm. The limit of detection and quantitation were calculated using the following equations: 3=signal to noise ratio and 10=signal to noise ratio. The limit of detection was 5ppm and the limit of quantitation was 17ppm. The curve had a slope of 157.9 abs. ion count/ppm, an intercept of -66.80 abs. ion count, and a R^2 value of 0.9850. The three

pure vanillas were tested against the curve using a bracketed quantitation technique were determined to have concentrations of 92±23ppm, 28±5ppm, and 1357±246ppm.

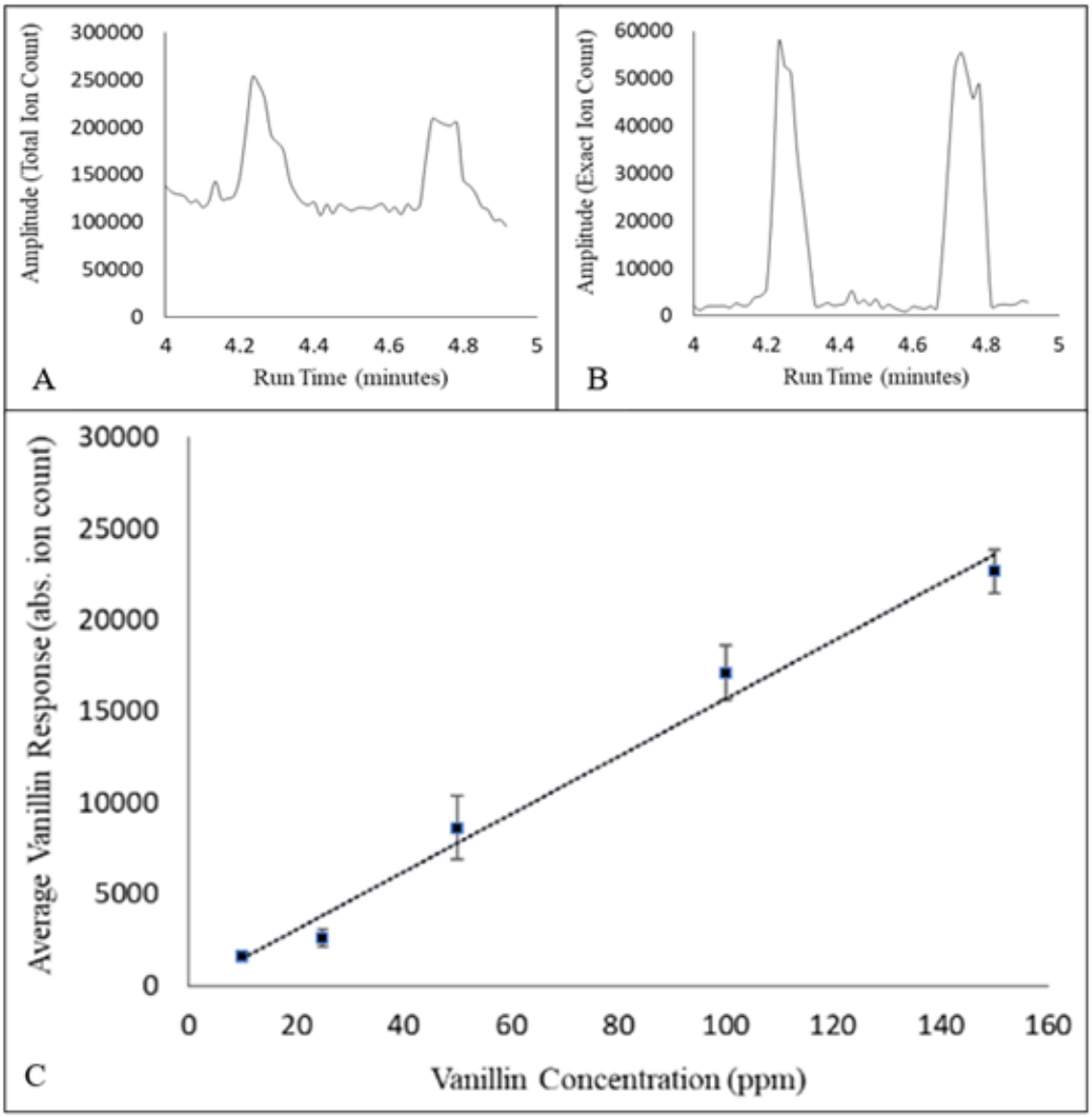


Figure 3.5. A) TIC of 2 of 3 150ppm standards B) EIC of 2 of3 150ppm standards C) Mass intensity calibration curve for vanillin ranging from 10 to 150ppm. The slope, intercept and R^2 values are 157.9 abs. ion count/ppm, -66.80 abs. ion count, and 0.9850, respectively.

3.3. HPLC Characterization

HPLC is a commonly used technique for quantitation and has been used to quantitate vanilla extracts. A HPLC method was developed to quantitate the store-bought vanilla extracts and was used as a comparison to the MIDAS quantitation method. The chromatography method served as guide to determine the quantitation capabilities of the MIDAS. The method was developed based on previously established vanilla extract methods. A standard curve ranging from 5 to 100ppm was constructed and yielded a slope, intercept, and R^2 value of 122,800 mAu/min/ppm, 214,800 mAu/min and 0.9999 respectively (Figure 3.6). It was determined that the vanilla concentrations were 639.9ppm, 474.6ppm, and 5118.5ppm. The limit of detection was calculated to be 2ppm and limit of quantitation was 7ppm, respectively.

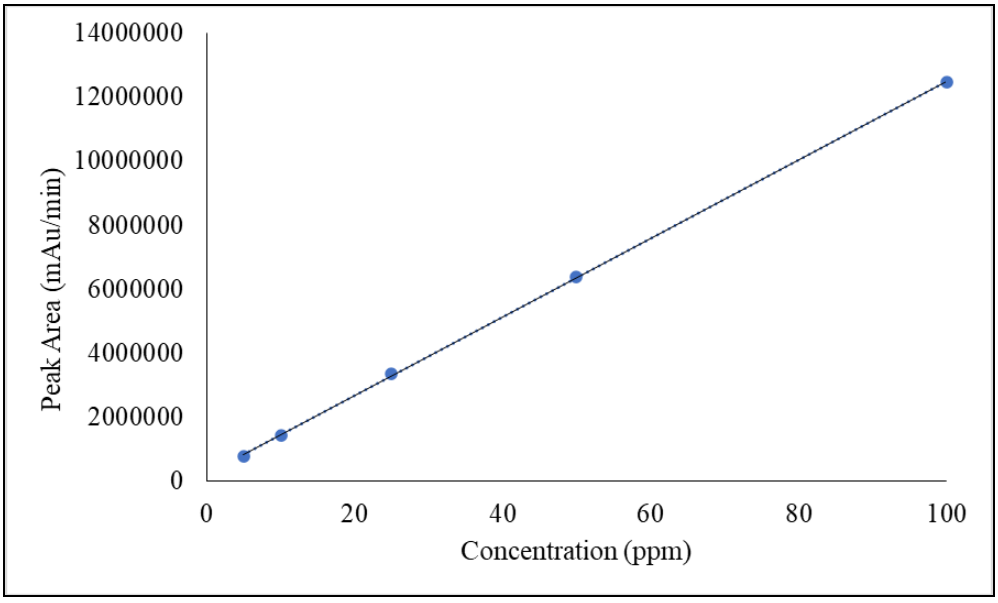


Figure 3.6. HPLC calibration curve for vanillin ranging from 5 to 100ppm. The slope, intercept and R^2 values are 122,800 mAu/min/ppm, 214,800 mAu/min, and 0.9999, respectively.

Comparing the HPLC method to the MIDAS there are some similarities that are shared between the two methods. The linear ranges are similar between the two methods with the liner range of 10ppm-150ppm for the MIDAS and 5ppm-100ppm for the HPLC method. The limit of detection and limit of quantification of the HPLC method is slightly lower than that of the MIDAS. Lastly, the trend of the detected concentrations is the same.

Figure 3.7 compares the vanillin concentrations that were yielded from both methods using a bracketed quantitation method. The concentrations yielded from the MIDAS method are significantly lower the HPLC. However, the trend of calculated concentrations is the same. Both methods calculated that the third pure vanilla contained more vanillin and the second one contained the less. Based on the concentration results, limit of linearity, and correlation coefficient, the MIDAS can be used as a semi-quantitative technique in conjunction with its strong qualitative output. MIDAS is internally reproducible, but the ionization mechanism causes some hinderance to quantitation. An internal standard in the case of the liquid extracts could be utilized to better the quantitation. Using mass spectrometry, there are many options for internal standards and one option could be the use of isotopes. Using an internal standard for powders is more complex. An internal standard would have to be spiked into a powder and then efficiently mixed throughout to ensure uniformity.

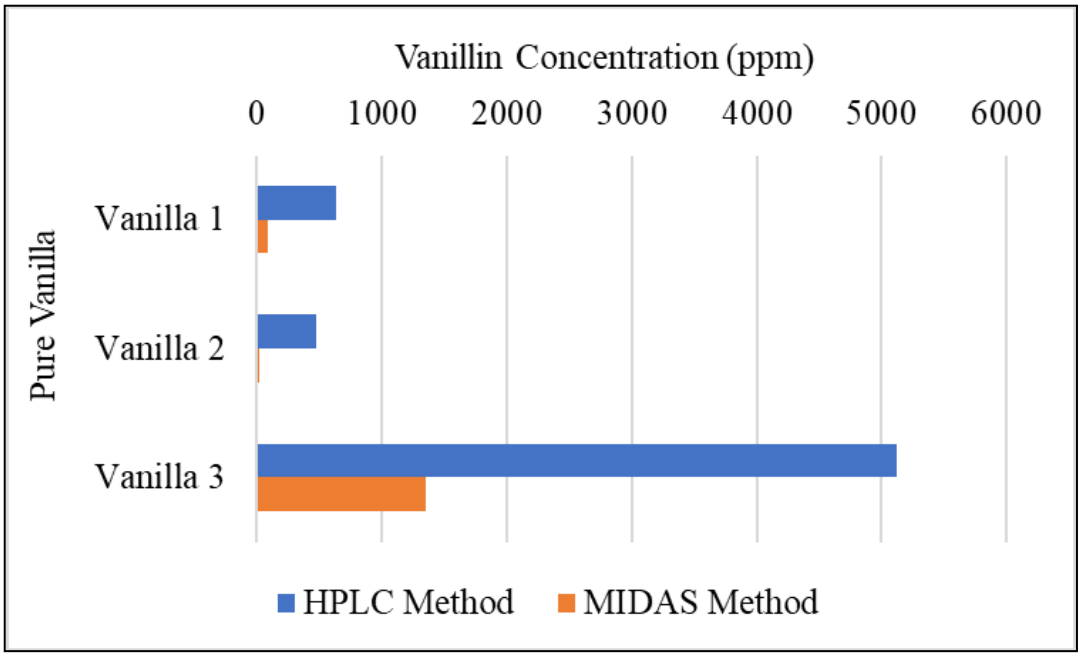


Figure 3.7. Concentration comparison between HPLC and MIDAS methods.

The result for the MIDAS is expected, due to the analysis conditions. First, ambient sources are open sources and are subjected to constantly changing conditions such as humidity change, temperature change, and other ambient compounds. These factors can influence the desorption and ionization process creating variances in analyte signal. To alleviate this effect, a closed source can be constructed to regulate these conditions. Another potential explanation for the difference, is the nature of the desorption and ionization process. The surfaces of the sampling platforms are not homogenous which could cause some hinderance to the desorption process. There is also a potential of losing sample during the desorption. One hypothesis is that the ambient conditions could affect the desorbed sample by hindering the desorption process (humidity) or preventing the desorb ions from making it to the MS (air currents). Another hypothesis is that sample surface is two dimensional and therefore the sample is heterogenous which could cause variances in signal intensity. Further

comparisons to other methods such as HPLC with atmospheric pressure chemical ionization can also be used to better understand the quantitation levels of the MIDAS. This concept is outside of the intent of the research but can be explored further in future work.

When compared to similar ambient ionization sources, the MIDAS is a more sensitive technique. Figure 3.8 displays a comparison between the DSA (B) and the MIDAS (A). Vanilla compounds have an increased sensitivity using the MIDAS and was able to detect present compounds that the DSA could not.

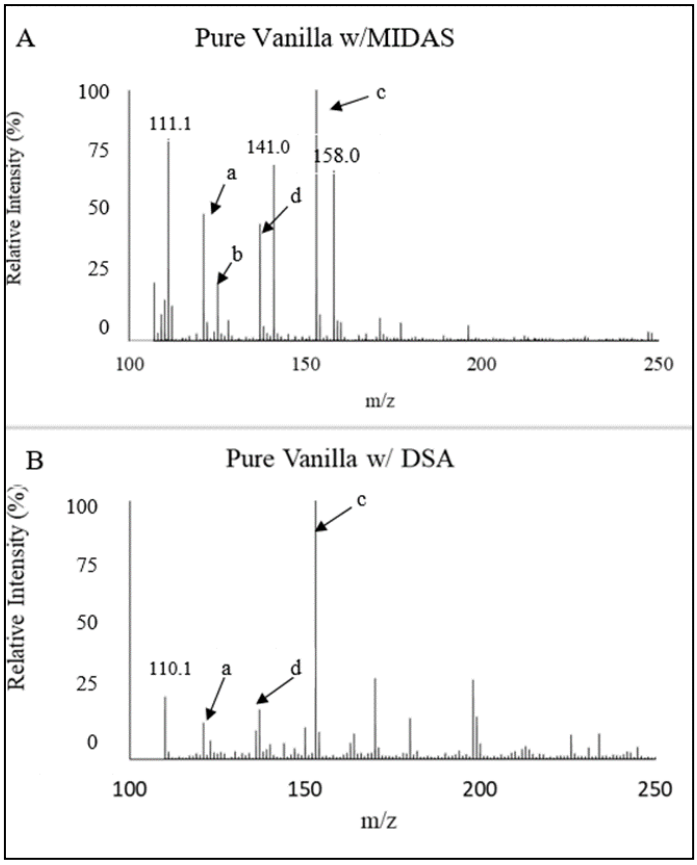


Figure 3.8. A) Pure vanilla 1 analyzed by MIDAS and B) pure vanilla 1 analyzed by DSA.

3.4. Coumarin Detection

After analyzing the extracts, there was no indication of adulteration. Therefore, coumarin was spiked into pure vanilla 3 (chosen at random) to simulate a coumarin addition. Spiked samples contained between 0.01% and 0.75% (v/v) coumarin to determine the limit of detection for this compound. Coumarin was detected in all the spiked samples with a linear response down to 0.01% (v/v) or 100ppb. The ratios of coumarin to vanillin are displayed in Figure 3.9 with a slope of 756.3abs. ion count/ %v/v, an intercept of 15.16 abs. ion count, and a R^2 of 0.9984. The limit of detection is 0.01% and the limit of quantitation is 0.04%. The inset in Figure 3.9 displays the ratio of coumarin to vanillin at 0.25%. The method can be used for detecting coumarin in samples and can be used for quality control of extracts that are produced in other countries.

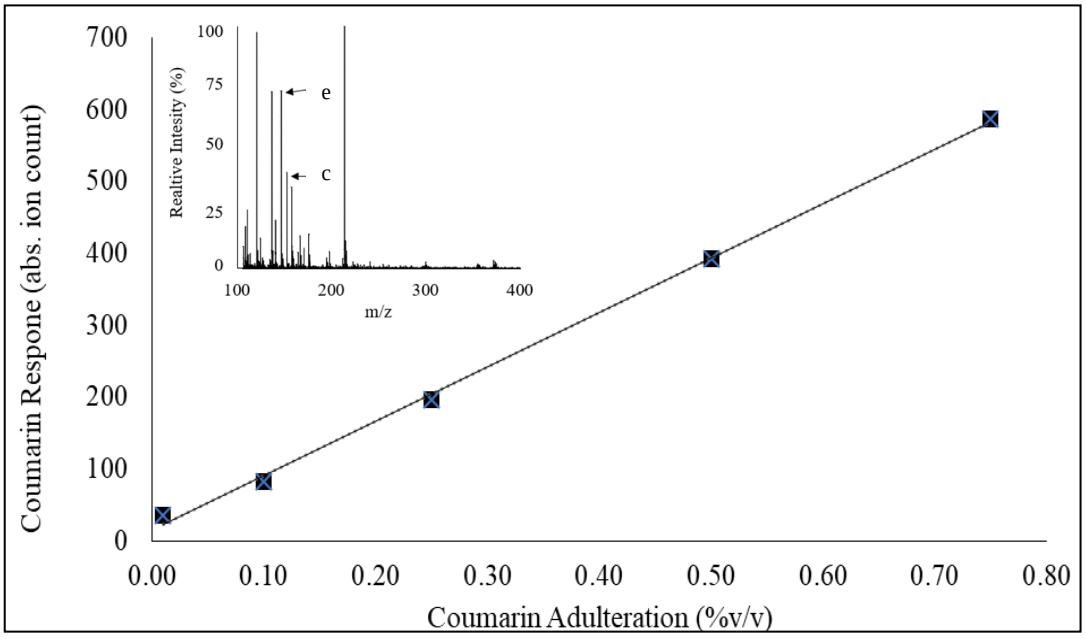


Figure 3.9. Coumarin was spiked into pure vanilla from 0.01% to 0.75%. The inset displays the ratio of (c) vanillin to (e) coumarin at 0.25% spiked coumarin. The slope is 756.3, intercept of 15.16, and R^2 of 0.9984.

3.5. Vanilla and Tonka Bean Analysis

Whole vanilla bean fragments were assayed to identify vanilla compounds in beans from known countries compared to extracts purchased from stores that did not disclose the origin. Extracts were made from the bean fragments and were assayed using the same method as the store purchased extracts. The solid bean fragments were the focus of this section to display the versatility of the MIDAS to be used for solid sample analysis. The solid plant matrix represents a paste like texture which is considered a more complex matrix. Vanilla beans from India, Indonesia, Madagascar, Mexico, Sri Lanka, Tahiti, and Tanzania were assayed along with tonka beans, which are used as a common adulterant source for vanilla extracts.

The new sample type spurred the creation of a new sampling plate that utilizes adhesive strips. The original analysis of beans on the 96-well plate posed an issue with sample stabilization under the gas stream. There was a need to stabilize the solids and fasten them to the sample platform. Current sampling platforms did not have the capability. The glass plate with adhesive secured the beans under the gas stream for successful ionization.

The use of tonka beans is mostly seen in vanilla extracts from Mexico[18]. Tonka beans are a natural source of coumarin but have vanilla like taste and smell and can be used as a vanilla additive. Figure 3.10 displays the differences in the Mexican and Tonka bean spectra. The tonka bean spectra can easily be distinguished from the vanilla because there are no common peaks and the presence of coumarin is in the

Tonka and were not detected in the Mexican. If tonka beans were to be used to enhance vanilla products it would be easily distinguished using this method.

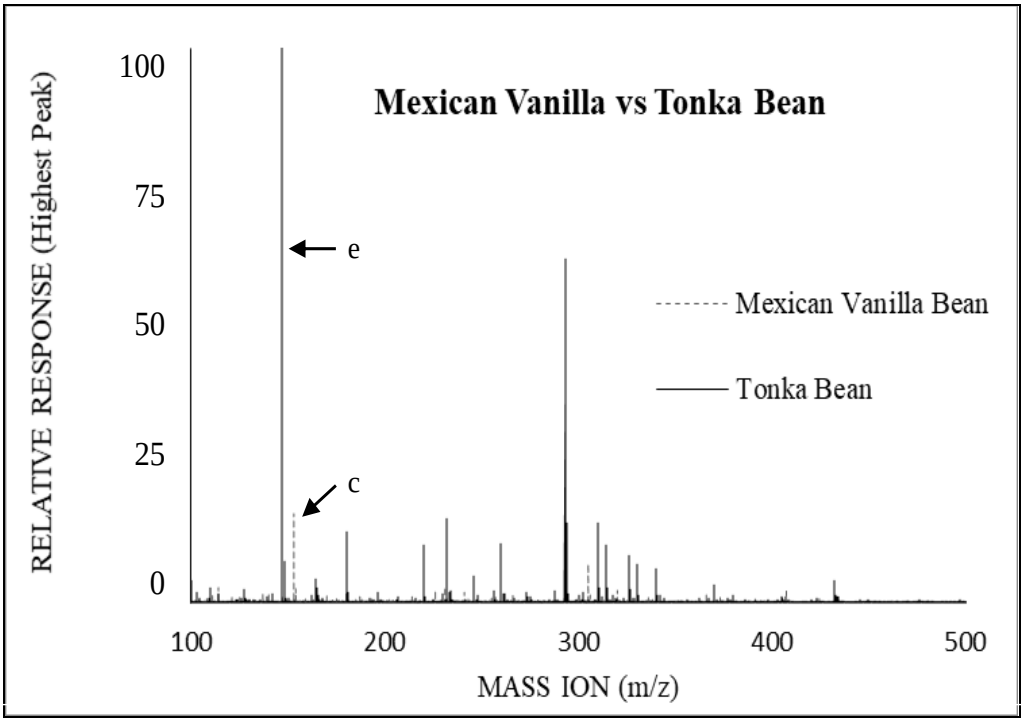


Figure 3.10. Mexican vanilla bean spectra and Tonka bean spectral overlay. Significant peaks include (c) vanillin($[M+H]^+$=153.1) and (e) coumarin ($[M+H]^+$=147.0).

Figure 3.11 displays the spectra of the different solid vanilla bean origins with vanilla compounds identified throughout the spectra. The extracts shared comparable results to those of the beans. Vanillin's intensity varies between countries and could be an indication of origin. The spectra for the Indonesian and Tahitian vanillas display 4-hydroxybenzoic acid (121.0) as the largest vanilla peak instead of the vanillin peak. Tahitian vanilla is expected to have a lower vanillin content because Tahitian vanilla is known to be more bitter and used for fragrances rather than confectionary products. The method has potential to be used as a country of origin identifier. There are also

differences in intensity of the vanillin peak as well unique masses associated to each country. The Indian, Madagascan, and Sir Lankan spectra have the same vanilla compounds present and peaks at 407.6 and 434.1. These compounds are thought to come from the bean pod itself, but further investigation is needed to confirm this hypothesis. The difference between the three are highlighted at the lower mass range (100-150) and the intensity of the mass cluster around the 407.6 and 434.1 peaks. The 407.6 and 434.1 peaks are lower in intensity in the Indian and Sri Lankan spectra compared to that of the Madagascan. The Sir Lankan spectra has less mass ion peaks throughout the spectra and the simplicity of the spectra with the identification of the three vanilla compounds could be used as a country identifier. The Tanzanian spectra has peak clusters at 407.6 and 434.1 but is missing the presence of vanillyl alcohol (d). Tahitian has vanillyl alcohol but does not have the 4-hydroxybenzyl alcohol peak (a) like all the other vanilla beans. The Tahitian spectra also has the 4-hydroxybenzoic acid which is only seen and is the most intense in the Tahitian and Indonesian vanilla. The distinguishing peaks between the Tahitian and Indonesian vanilla is that the Indonesian contains all four of the main vanilla compounds and has an intense response at 407.6 compared to the other vanilla spectra. This spectral difference could be potentially used to identify country of origin.

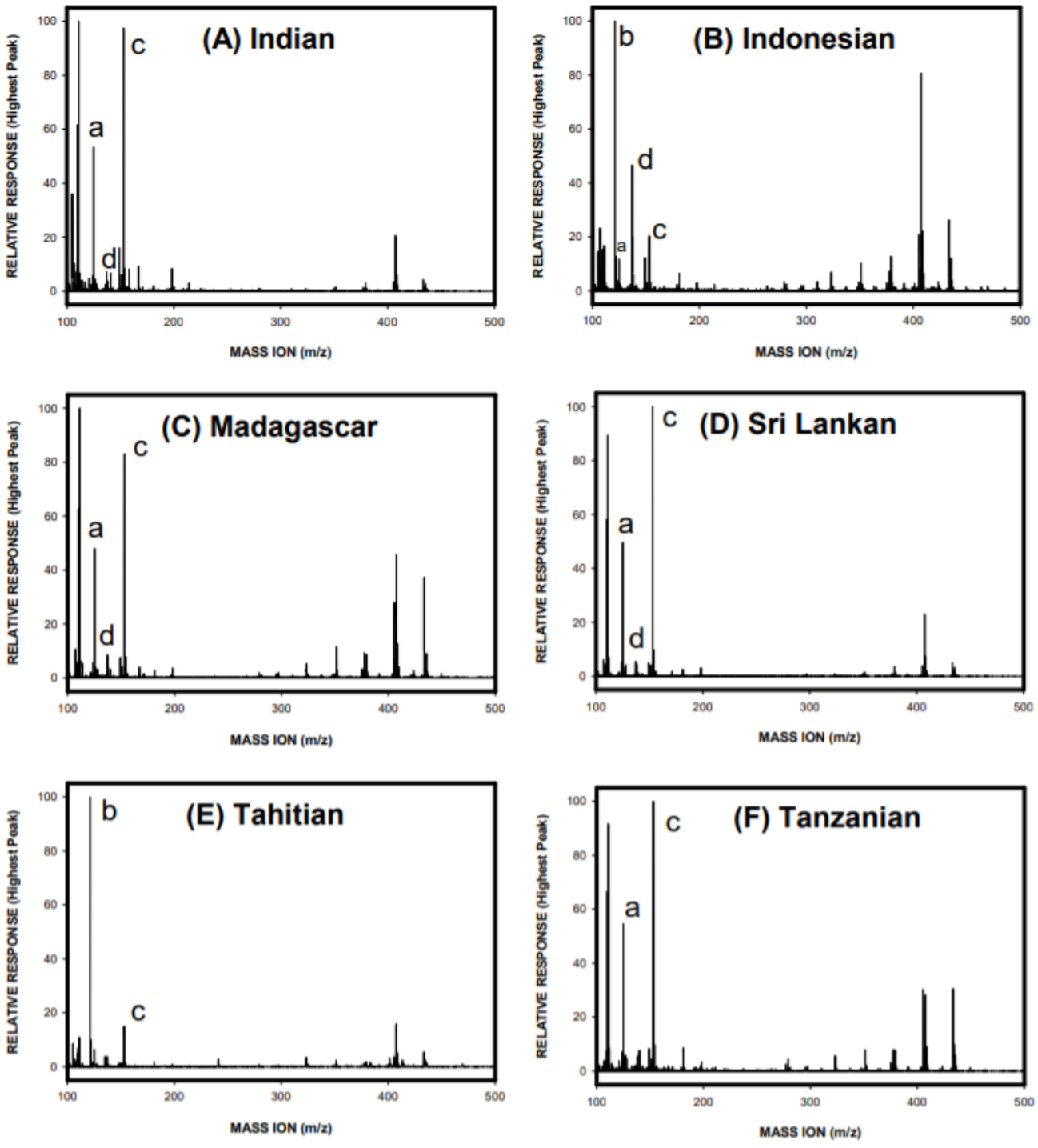


Figure 3.11. Country of origin comparison. Significant peaks (a) 4-hydroxybenzyl alcohol ($[M+H]^+$=125.1), (b) 4-Hydroxybenzoic acid ($[M-OH]^+$=121.0), (c) vanillin ($[M+H]^+$=153.1), (d) vanillyl alcohol ($[M-OH]^+$=137.1).

3.6. Conclusion

MIDAS-TOF-MS can rapidly characterize both vanilla extracts and bean fragments making MIDAS suitable for both liquid and solid samples. Vanilla compounds were detected in all pure vanilla samples with no signs of adulteration present, and imitation vanilla extracts contained the expected ethyl vanillin peak. Spiked coumarin samples were linear down to 0.01% (v/v) giving the method a limit of detection of 100ppb. MIDAS is internally reproducible and has quantitative properties that would be complementary to HPLC analysis. MIDAS can rapidly identify compounds with internal consistency. Unfortunately, the absolute value of a compound (e.g., vanillin) in a sample is not consistent with known methods due to effects of the ionization/desorption process and potential matrix effects. However, MIDAS is still an invaluable analytical technique for the rapid identification of compounds and the reproducible fingerprinting of a wide array of samples, especially solids.

A new sampling plate was designed to stabilize solid samples under a gas stream. Assaying beans from various countries showed how MIDAS can be easily used for large, irregular shaped solids that have caused a hindrance in existing ambient techniques. MIDAS shows the potential to be used in further vanilla bean analysis and could be a useful tool in distinguishing country of origin. Lastly, assaying beans from various countries showed how MIDAS can be easily used for large, irregular shaped solids that have caused a hindrance in existing ambient techniques. MIDAS shows the potential to be used in further vanilla bean analysis and could be a useful tool in distinguishing country of origin.

Chapter 4: Characterization of Black Peppercorn

4.1. Introduction

Piper nigrum (black pepper) is one of the most used spices due to its aroma and pungent flavoring. Commonly used as a flavoring for food and beverages, black pepper can also be used for perfumes and medical use[20, 21]. Piper nigrum has been used clinically to treat stomach ailments and is known to possess both antioxidative and anti-inflammatory properties. Popularity of the spice as flavoring and alternative medicine has increased the demand for higher quality products. Increase in demand has increased the risk of black pepper products to contain additives. Some common adulterants of black peppercorn products are papaya seeds, mineral oils, other strands of pepper, other seeds and berries, spent black pepper corns, and stems of black pepper plants[2, 6, 22]. Black pepper powders are commonly adulterated with dyes, papaya seed powder, other seed and berry powders, starch, monosodium glutamate, other parts of the berry, and chili powder[2, 6]. Papaya seeds are the main additive for whole black peppercorns because their likeness in size and shape.

This chapter will display the versatility of the MIDAS to be used for solid spice product analysis. Whole and ground peppercorns will be analyzed using different sample platforms. Piperine, the main compound in black pepper will be compared both between whole and ground samples. In addition to piperine intensity, papaya seeds will be assayed, and the spectra will be compared to those of the peppercorns.

Samples that contain peppercorns and papaya seeds will be assayed to display the qualitative ability of the MIDAS to distinguish between black pepper and papaya.

This chapter will also demonstrate the MIDAS's versatility across several sample types. Assaying both whole and powdered solids highlight abilities that the MIDAS possess over other ambient techniques. New sampling platforms have also been created to assist in the navigation of assaying different sample types.

4.2. Results and Discussion

4.2.2. Black Pepper Corns

Black peppercorn is one of the most used spices and therefore rapid characterization and detection methods are needed to ensure product quality. Previous literature has utilized Direct Analysis in Real Time (DART) MS to characterize and compare different *Piper* species. However, the comparison of whole and ground *Piper nigrum* (black pepper) has not been discussed. When assaying the whole corns, the adhesive plate crafted in chapter three for the vanilla beans had some setbacks. The whole corns would not adhere to the plate and would blow off when under the nitrogen stream. This caused a hinderance to the proper ionization of the black peppercorns. Figure 4.1 shows the new plate that was created to aid the ionization process of the whole corns. The adhesive rug tape secures the corns to the sampling plate without interfering with the ionization. This plate was used for both the whole peppercorns and papaya seeds. The ground samples were assayed using the original adhesive platform.

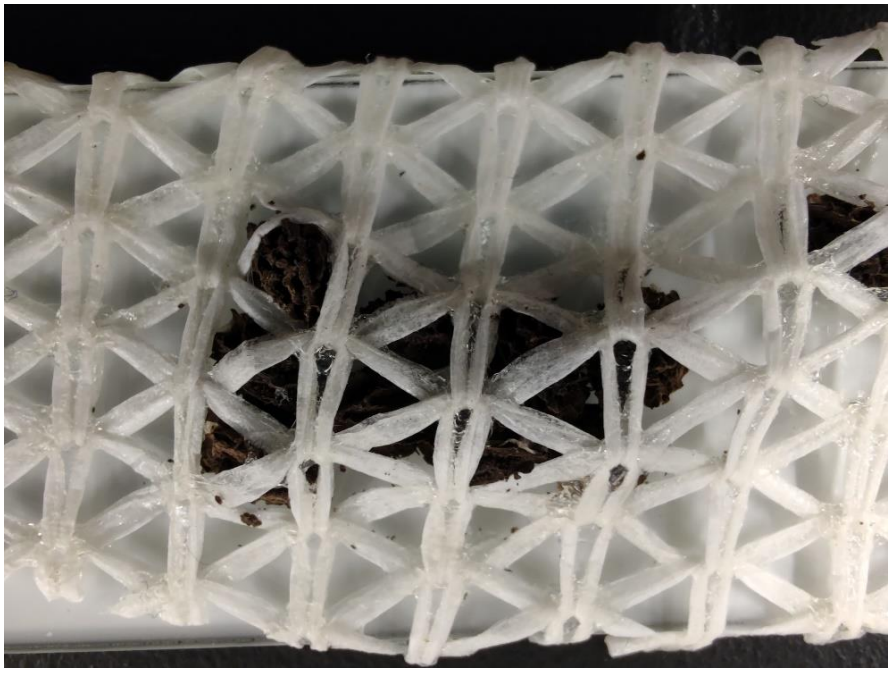


Figure 4.1. Sampling platform for whole corns and seeds.

Figure 4.2 displays a comparison of two types of whole black peppercorns from different growing regions labeled “B” and “V.” The most prominent peaks occur at 286.1 ($[M+H]^+$) and 571.2 ($[M+H]^+$) which correspond to piperine and dipperamide A, respectively. Piperine is the main compound always present in black peppercorns and can be used as an identifier for pepper samples. Dipperamide A is also a common compound that is commonly seen in black pepper and has been characterized in literature.

The overlay of the two peppercorns shows similarities between the two different whole corns. The notable differences are the intensities of the piperine and dipperamide A, where peppercorn V(orange) has a higher peak intensity. This may be an indication of the concentration difference between the two different corns. However, the trend reversed when both corns were ground.

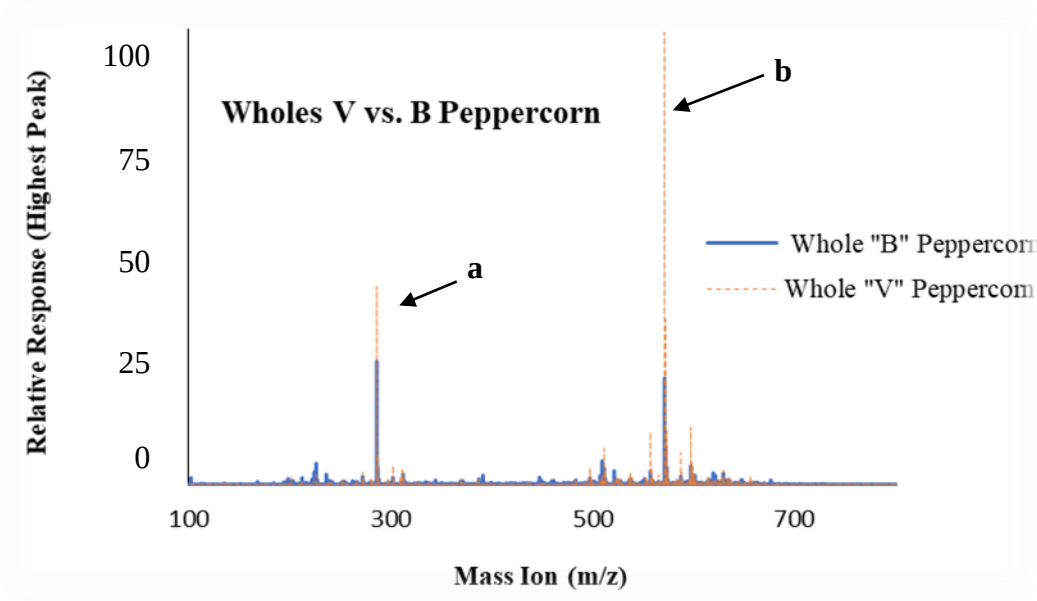


Figure 4.2. Comparison of two different types of whole black peppercorns from different growing regions labeled "B" and "V." A) 286.1 ($[M+H]^+$) and B) 571.2 ($[M+H]^+$) correspond to piperine and dipperamide A, respectively.

The piperine and dipperamide A peaks were at a lower intensity in the ground V corn than the B. This may be an indication that the pepper compounds are more concentrated in the inner parts of B compared to those of V which may be evenly dispersed throughout the corn. Figure 4.3A displays an example of the ground and whole V corn spectra. The piperine intensity does not change much between the ground and whole version of the peppercorn which may indicate the uniformity of the compound throughout the corn. Dipperamide A does decrease about 25% from the whole to the ground sample which may indicate that it is more prevalent on the surface of the peppercorn. Peppercorn B (Figure 4.3B), however, had a drastic difference between the whole and ground sample. Both compounds, with an emphasis on dipperamide A, displayed larger intensities in the ground samples which is an indication that the compounds are more concentrated towards the inside of the corn

for the B sample.

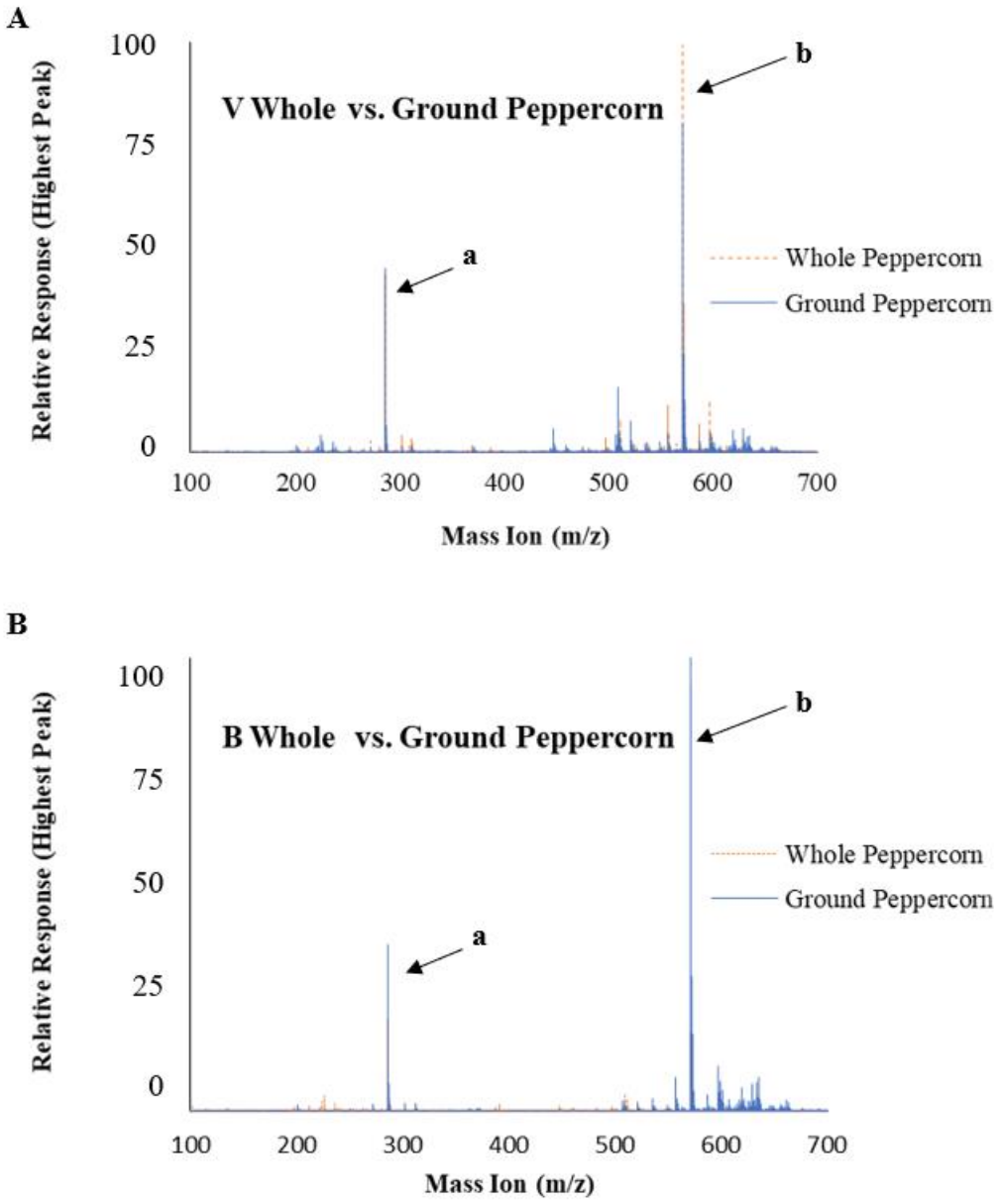


Figure 4.3. Whole and ground peppercorns for B and V overlayed. A) 286.1 ($[M+H]^+$) and B) 571.2 ($[M+H]^+$) correspond to piperine and dipperamide A, respectively.

4.2.3. Papaya

Papaya analysis has been mostly limited to the pulp of the fruit and not the seeds. Whole and ground papaya seeds have not been fully explored in the literature and the

exploration has been limited to crude products and oils. Figure 4.4 shows the overlay of the spectra of whole and ground papaya samples. There is a distinct difference between ground and whole papaya samples and is a different trend than what is seen in the pepper samples. The difference between the spectra is indicative of the compounds concentrated inside. In the whole seed spectra, there is signal for benzyl isothiocyanate (MW 149.2) which is one of the main components found in papaya. The presence of the compound in the whole seed versus the ground indicates that it may be limited to the fruit of the papaya and not necessarily the seeds or that the signal is diluted in the ground form. Papaya compounds have not been well characterized in the literature and the spectral peaks are used a fingerprint identifier. Differences between the papaya seed spectra are important to note when comparing papaya and peppercorn samples. Whole and ground samples will be analyzed separately in the next section.

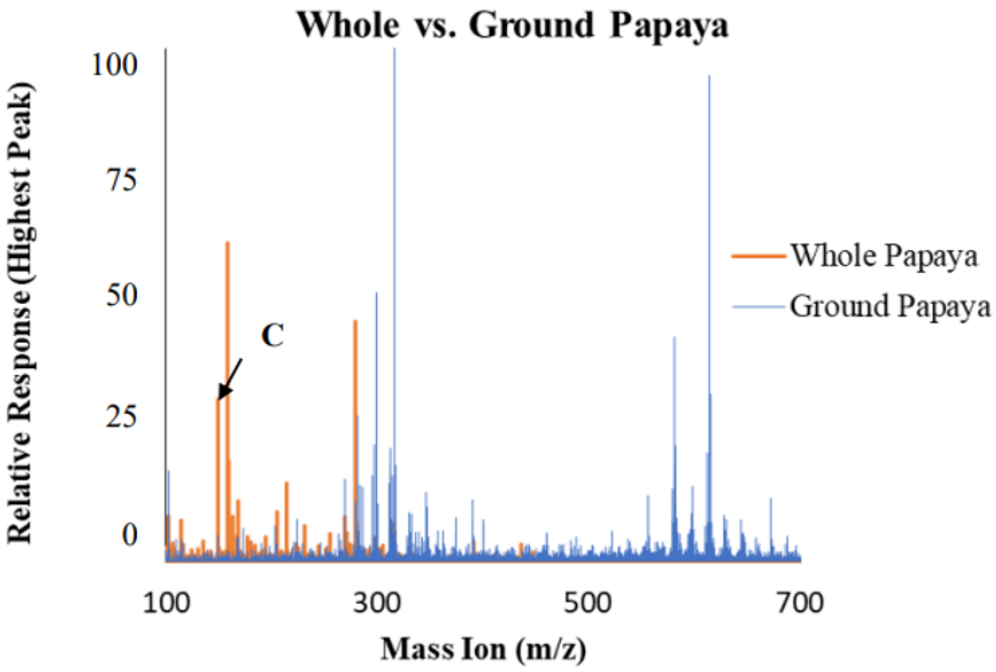


Figure 4.4. Whole and ground papaya overlayed. C) Benzyl isothiocyanate (149.0 ($[M]^+$)).

4.2.4. Pepper vs. Papaya

As stated in the previous sections, pepper and papaya are similar in appearance and a quality control method is needed to distinguish between peppercorns and papaya seeds. Figures 4.5 and 4.6 are a spectral overlay of a peppercorn (blue) and papaya seeds (orange). There are little to no mass overlaps seen between the two spectra for the whole corns and seeds (Figure 4.5). Piperine and dipiperamide are detected in the peppercorn spectra and are absent of in the payas and benzyl isothiocyanate is in the papaya but does not appear in the pepper. If papaya is added to whole black peppercorn samples, spectral data alone can identify the presence of papaya.

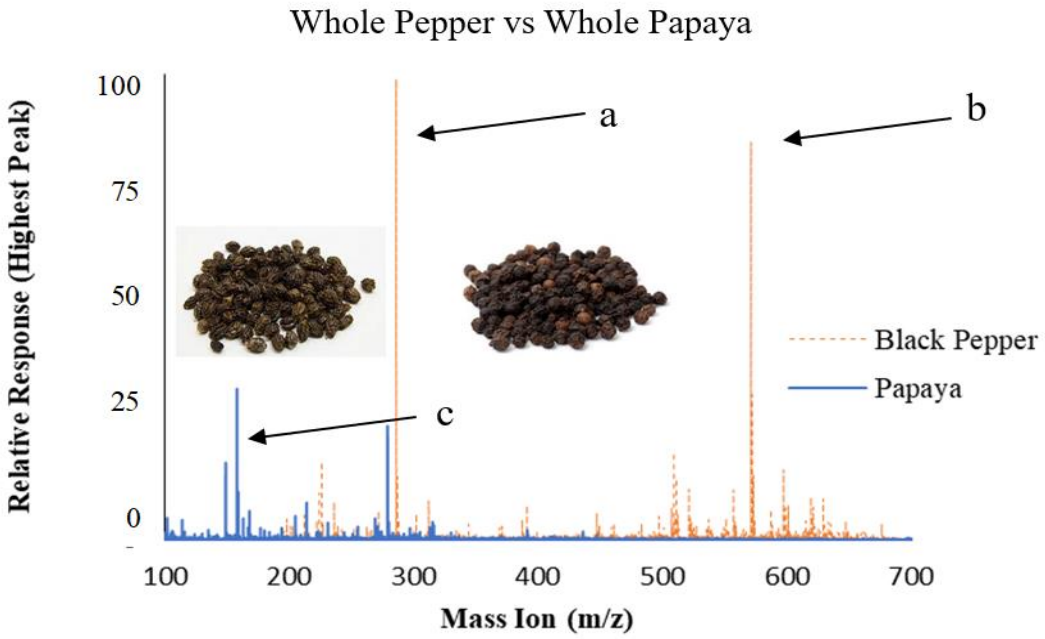


Figure 4.5. Whole peppercorns and papaya overlayed. A)piperine, b) dipiperamide, c)benzyl isothiocyanate

Since there is a distinct difference between whole and ground papaya seeds it would be easier to differentiate the two, however, there is not a distinct difference between ground papaya and ground pepper. Figure 4.6 shows the spectral overlay of the

ground papaya and black pepper samples. In the ground papaya sample, there are three distinct masses that are prominent in all the ground papaya samples. Masses 316.3, 581.5, and 614.5 are seen in every ground papaya sample and are not present in the peppercorn spectra. These masses can be used as ground papaya markers when analyzing unknown samples. These masses are unknown and could be indicative of compounds present in the papaya seeds that have yet to be characterized.

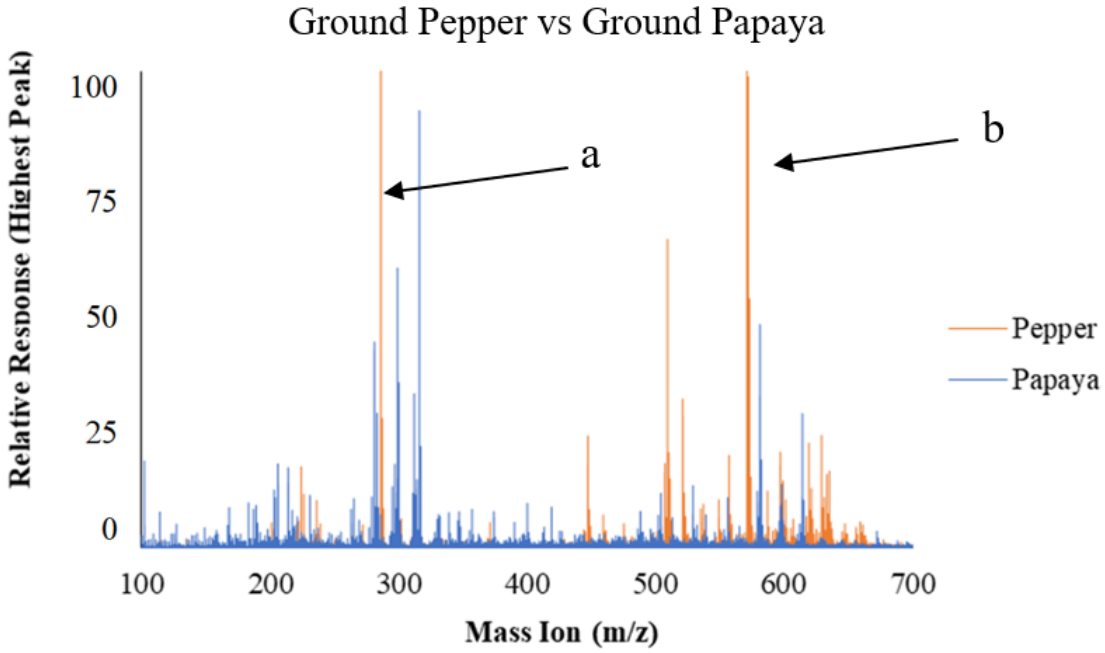


Figure 4.6. Ground peppercorns and papaya overlayed. a)piperine and b)dipiperamide

A two-sampled Kolmogorov-Smirnov and a Z test was performed on the pepper and papaya data and confirmed that papaya was statistically different from that of black peppercorn. The two-sampled Kolmogorov-Smirnov (KS) test is used to evaluate samples similarity to each other. Compared to the t-test, the KS test can compare large multivariable data. The t-test assumes that the average outcome of one set of data is from the average outcome of another set and therefore may not be able to

determine the differences in more complex data sets. The KS test does not make assumptions about the distribution of the data in the data set, but instead evaluates the entire data set. In the KS test the entirety of the two samples are evaluated and the null hypothesis would state that “sample 2” does come from “sample 1”. Rejecting or accepting the null hypothesis is based on if the maximum difference is larger or smaller than critical difference (D crit). In a two sample KS test “D crit” is calculated using equation 1. N_x and n_y are the number or observations found in each of the samples.

$$D_{crit,0.05} = 1.36\sqrt{\frac{1}{n_x} + \frac{1}{n_y}}$$

Based on the visual differences and the information gained form the KS test, the ability of the method to distinguish between papaya and black peppercorns was confirmed. When testing blind samples, the MIDAS can distinguish between whole papaya seeds and whole peppercorns. The spectra are distinctly different and benzyl isothiocyanate is detectable in the papaya spectra. When assaying ground samples, the MIDAS cannot easily distinguish between the two. There are some unique differences between the two spectra, but assistance statical assistance is needed to confidently confirm the differences. The benzyl isothiocyanate is not detected in the ground papaya and could be due to the location of the compound relative to the seed. The compound could be isolated to the exterior of the seed and when crushed, the

signal is diluted.

4.3. Piperine Quantitation

MIDAS quantitation was addressed in Chapter 3 with vanilla extracts on the 96-well plate. Quantitating piperine could be advantageous in differentiating between growing regions and types of peppercorns. This process would include extracting piperine out of the whole or ground peppercorns, which is out of the scope of this chapter.

Using the adhesive sampling plate mentioned for both vanilla beans and ground pepper, piperine standard was spotted and assayed. Figure 4.7 displays the linear range (5ppm-100ppm) of piperine on the adhesive plate. This is the same range that was observed by the HPLC method and like that of the 96-well plate. The slope, intercept, and correlation coefficient are 51.21 abs .ion count/ppm, 2135 abs. ion count, and 0.9917, respectively. The limit of detection and quantitation were 3ppm and 11pm, respectively. The technique has a strong correlation coefficient and suggests there are some quantitative capabilities. However, quantitation using an internal standard with solids is not a viable approach. Solids are used for fingerprinting and for identification. Quantitation would be better suited for liquid samples such as extractions.

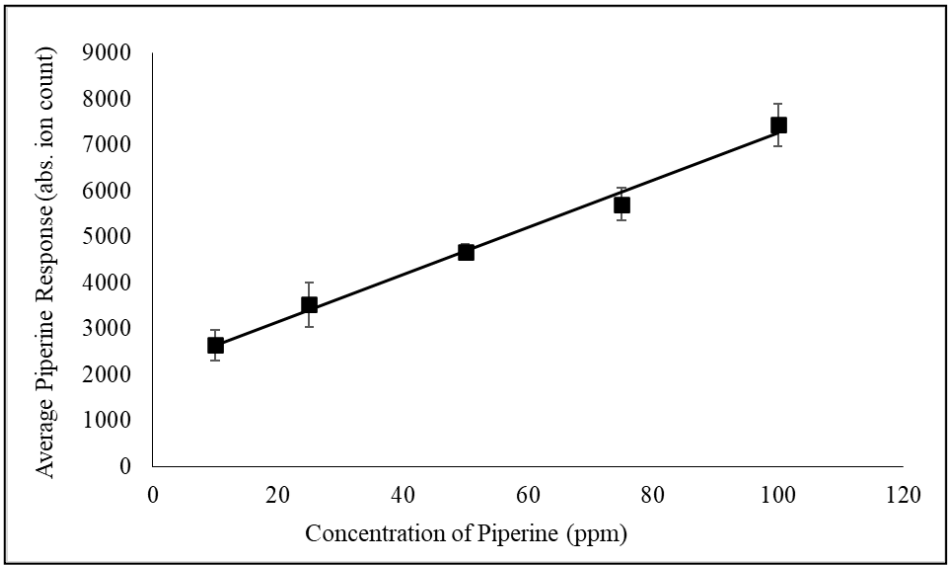


Figure 4.7. Piperine calibration curve on the adhesive plate ranging from 5 to 100ppm. The slope, intercept and R^2 values are 51.21 abs. ion count/ppm, 2135 abs. ion count, and 0.9917, respectively.

To simulate a more accurate representation of piperine in peppercorns, a cotton swab was used to explore quantitation of piperine. The surface of the cotton swab has more of a porous texture and better represents the surface a peppercorn. Therefore, the piperine standard was assayed to yield a linear range of 0.5ppm to 10ppm. Figure 4.8 displays the curve with a slope, intercept, and correlation coefficient of 2981 abs. ion count/ppm, 8208 abs. ion count, and 0.9546, respectively. The limit of detection was 0.3ppm and the limit of quantitation was 0.8ppm. Utilizing the swab, increased the limit of detection by ten-fold. This may be indicative of how the sample interacts with the swab compared to the smooth surfaces. The correlation is not as strong as the other two plates. The sampling depth of the swab compared to the smooth surfaces may be the cause. Since the sample can soak further into the swab, the desorption and ionization process may be different compared to the smooth surfaces.

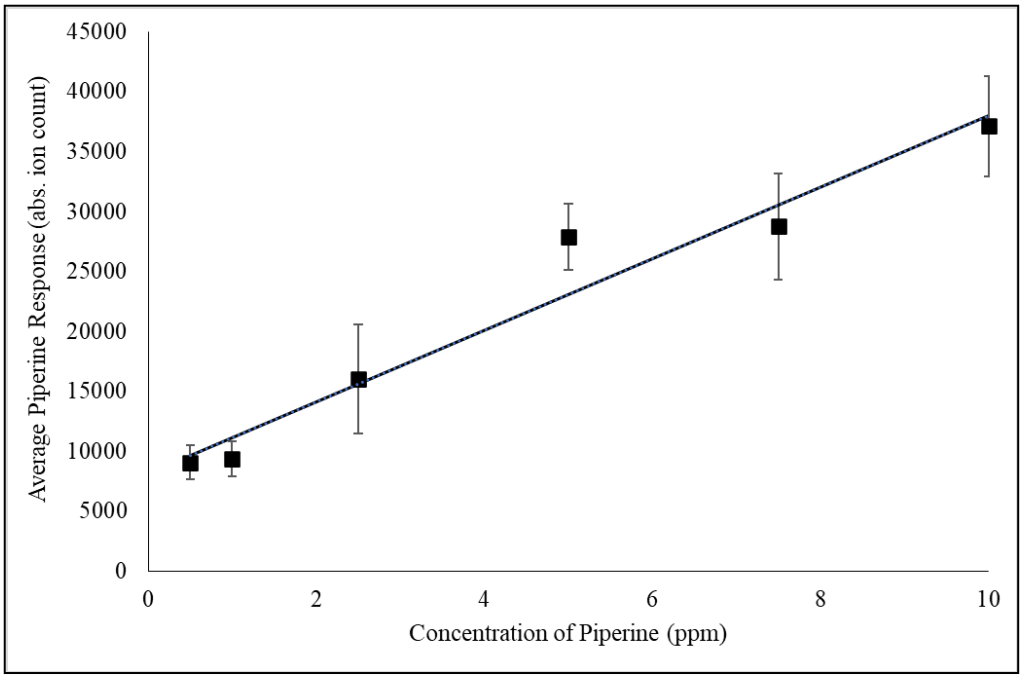


Figure 4.8. Piperine calibration curve on cotton swabs ranging from 0.5 to 10ppm. The slope, intercept and R^2 values are 2981 abs. ion count/ppm, 8208 abs. ion count, and 0.9546, respectively.

4.4. Conclusion

Major compounds for black pepper were detected in the two different peppercorns tested. The differences between ground and whole peppercorns give indication that compounds may be dispersed throughout the corns differently. V had compounds more evenly dispersed whereas B had compound more concentrated in the inside of the corn.

Papaya is one of the most common adulterants for black peppercorns due to visual similarities. MIDAS can characterize both peppercorns and papaya seeds and can easily distinguish the differences in the spectral data between the two. Using a two-sampled Kolmogorov-Smirnov and a Z test, the differences between papaya and black peppercorns were confirmed. Spectral and statistical differences provide

confirmation that the MIDAS can be used to rapidly differentiate black pepper from papaya.

Chapter 5: Exploration of Drug Detection in Synthetic Ear Wax

5.1. Introduction

MIDAS has been used for food adulteration and is has displayed the ability to analyze solid and liquid samples. The versatility of the MIDAS can be applied to other fields such as forensic analysis. Forensic analysis can give insight on biological processes. For instance, different compounds can be detected to determine drug use and abuse[62-66]. Not only can the parent compound be detected, but the metabolites of that parent compound when it is processed by the body can be detected. Common secretions that are analyzed for drug detection include blood, urine, saliva, and in some instances sweat[62-67]. Hair, although not categorized as a secretion, has also been used to determine drug use. Recently, exploration of ear wax as a secretion used for drug detection has been explored[63-66, 68]. Ear wax is a unique secretion that is ideal for drug detection.

Ear wax is secreted by the body and is housed in the external acoustic meatus which is the passageway that leads to the outside of the ear[69]. Residing directly in front of the tympanic membrane, ear wax acts a protective barrier to the middle ear. As the barrier, ear wax collects all debris and contaminants that enter the ear including skin cells. Therefore, when analyzed, ear wax is compiled of about 60% keratin, 12-20% saturated and unsaturated long-chain fatty acids, alcohols, and 6-9% cholesterol[65, 70]. Since ear wax is a secretion and a barrier it can be used to test for drug use with both the parent compound and its metabolite present in the sample[64-66, 68]. The parent compound will be present in the ear wax before the metabolites. This is due to

the compounds being trapped by the “barrier” that the ear wax creates during the time of drug use. There can be a period where the parent compound is present, and the metabolite is not and vice versa. The metabolites will be present from the body’s natural secretion process and will more than likely be the target of drug research and therefore will be the focus of this project. In this chapter the exploration of nicotine and tetrahydrocannabinol (THC) metabolites will be the focus. The goal of this chapter is to explore the ability of the MIDAS to be used for assaying biological samples and expand upon sample versatility.

5.2. Detection Methods

Drug detection in ear wax has been a recent development and therefore detection methods are still emerging. Table 5.1 is a non-exhausted list of current detection methods for ear wax and a noncomprehensive list of analytes that were detected using each method. Most methods utilize mass spectrometry (MS) because of its sensitivity and selectivity of target analytes even in the most complex matrices[62, 64, 70-72]. Chromatographic techniques are utilized mostly for identifying drugs and their metabolites within the ear wax. Specific ear wax characterization was executed most by GC analysis to ensure detection of any volatile compounds present[70]. Polymerase chain reaction (PCR) analysis is dedicated to that of genetic testing[66, 73]. The target molecules are associated with biomarkers of various diseases, DNA, and RNA. This method is better suited for a medical exploration rather than that of a criminal. There are currently no other ambient techniques exploring ear wax analysis, and therefore using the MIDAS provides a novel detection approach.

Table 5.1. Non-exhausted list of ear wax detection methods

Detection Method	Analytes Detected
LC-MS/HRMS/TOF	MDMA, MDA, MDEA, methylphenidate, cocaine, benzoylecogonine, morphine, codeine, heroin, 6-MAM, methadone, EDDP, THC, nicotine, cotinine, opiates, cannabinoids
GC-MS	Organic components of ear wax (saturated and unsaturated long chain fatty acids, alcohols, squalene, cholesterol)
PCR	Hepatitis, DNA, RNA

5.3. Removal of Earwax

Ear wax that is located on the inside of the ear canal should be collected because it is shielded more efficiently from external contaminants such as shampoo residue. Collection methods use a removal apparatus to retrieve the earwax from inside the ear. The main sample collections techniques are with a cotton swab (most common), sterilized metal scoops, plastic curettes, wooden spatulas, and the Jobson-Horned probe[66, 70, 74]. Most of these techniques are performed by medical professionals to ensure the inner ear is not damaged. Samples that patients collect themselves are conducted with cotton swabs because the swab is efficient in collecting both dry and wet wax types. In a clinical setting the scoops are the most efficient for dry ear wax and the Jobson-Horne probe (Figure 5.1) is best for wet ear wax.

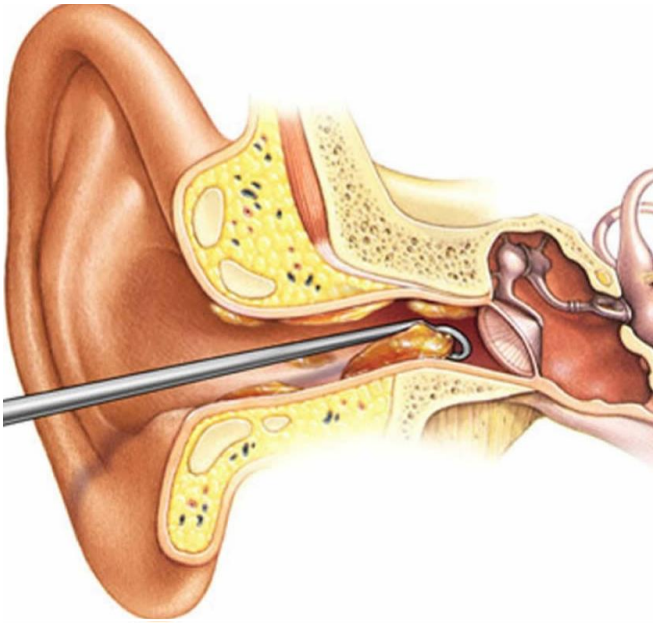


Figure 5.1 Schematic a Jobson-Horne probe removing ear wax from the ear.

After collection, the ear wax is placed into airtight sterile containers and kept away from sunlight, chemicals, and conditions that could cause sample degradation[70]. Preservation of samples is variable depending on the studies conducted. DNA testing conditions can be left at room temperature for up to six months after collection[70]. If volatile organic compounds (VOCs) are being analyzed, the samples should be stored in a -30C freezer and analyzed within a week of collection to prevent loss of analyte. Analysis of lipid omics and proteomics require the sample to be stored at -18C and -80C respectively[70].

5.4. Earwax Samples

Exploring ear wax as an application for the MIDAS started with testing of real ear wax. The purpose of this procedure was to test if the matrix were suitable for MIDAS analysis and if any compounds could be detected. Figure 5.2 shows the spectra for the wax collected out of the left and right ear three months apart. Two of the major

compounds in ear wax are cholesterol (C= 387.1($[M+H]^+$) and squalene (S= 411.0 ($[M+H]^+$) and were detected in all spectra. Other major peaks were not characterized but were hypothesized to be potential compounds associated with environmental factors or hygiene products that were trapped in the ear wax. The detection of these compounds indicates that ear wax has the potential to be analyzed by the MIDAS.

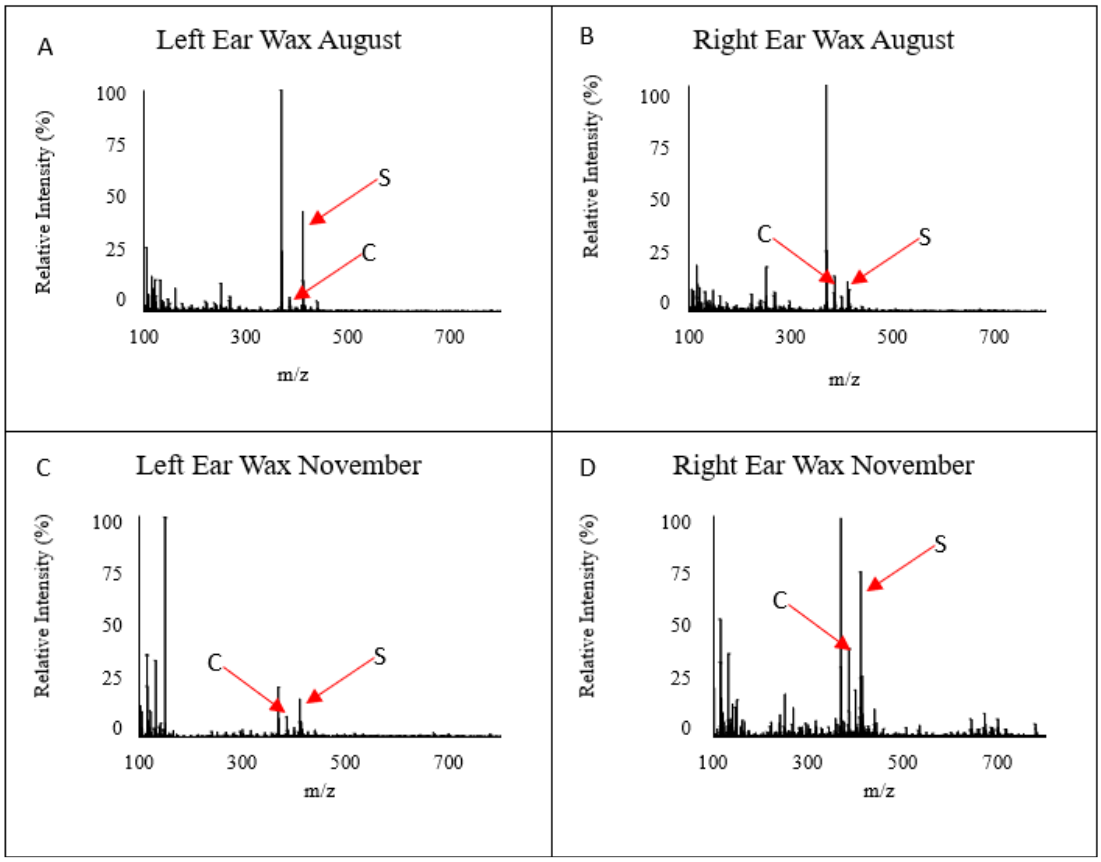


Figure 5.2. A) Left and B) right ear wax collected. Cholesterol (c) and squalene (s) detected. C) Left and D) right ear wax recollected after 3 months with cholesterol and squalene detected.

5.5. Synthetic Earwax

Collecting ear wax from human ears is considered slightly invasive and would require consent from volunteers. Ear wax from the ear is also consistently changing (seen in Figure 5.2) in content and would not be the ideal matrix to use for method development. Due to these reasons, synthetic ear wax is a suitable substitute to build a

detection method. Synthetic ear wax is made up of pure oleic acid, pure linoleic acid, anhydrous Lanolin, pure palmitic acid, Paraffin oils, and pure myristic acid. The synthetic ear wax is a viscous liquid that is ideal for spike analysis.

5.6. Introduction to Target Compounds

Exploration of drug metabolites in ear wax aid in the demonstration of MIDAS as a suitable analytical tool for identifying drug use. Common drugs that are used and occasionally abused are those of nicotine and tetrahydrocannabinol (THC). This research focuses on the metabolites associated with the use of these drugs and the detection limits associated with the metabolites in the ear wax. The focus of this exploratory project will be on cotinine, the metabolite for nicotine, and 11-hydroxy-THC, the metabolite associated with Δ9-tetrahydrocannnabinol.

Cotinine and 11-OH-THC were both detected in synthetic ear wax and are displayed in Figure 5.3. The cotinine standard gave a more intense response than that of the 11-OH-THC standard. This can be potentially contributed to the structural difference between the two compounds and the interactions between the earwax. The current detection platform uses single swabs and limits the throughput of the method. To increase the throughput of drug detection, a 96-well plate could be used for rapid drug detection. Figure 5.4 is a proposed diagnostic technique that can be implemented for rapid drug detection.

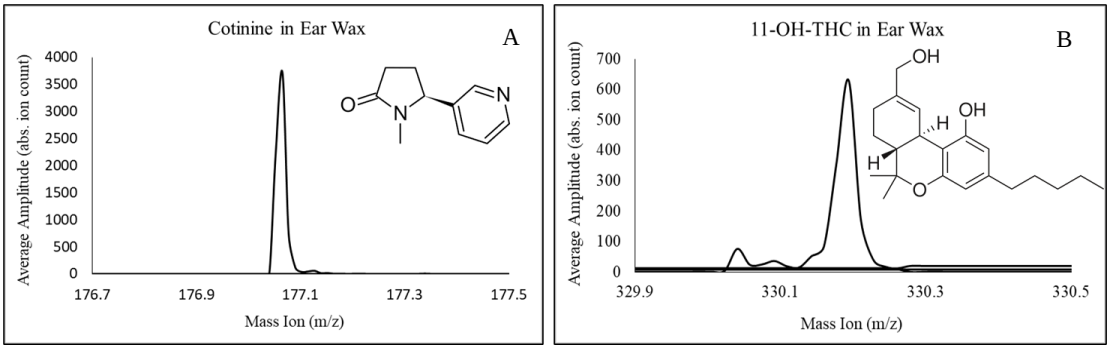


Figure 5.3. A) mass spectrum of cotinine in synthetic ear wax and B) 11-OH-THC in synthetic ear wax.

The proposed diagnostic technique would take a swab of ear wax and placed into ethanol solution. This technique is like sampling nasal swabs or throat swabs for various illness detection methods. The ethanol solutions aid in the extraction of the targeted compounds from ear wax matrix. The sample then can be pipetted onto a 96-well plate for a high throughput detection method. Testing one sample multiple times can also decrease the probability of fake positive or negative results. This technique can also be applied to other biological matrixes such as blood, urine, sweat, and spit.

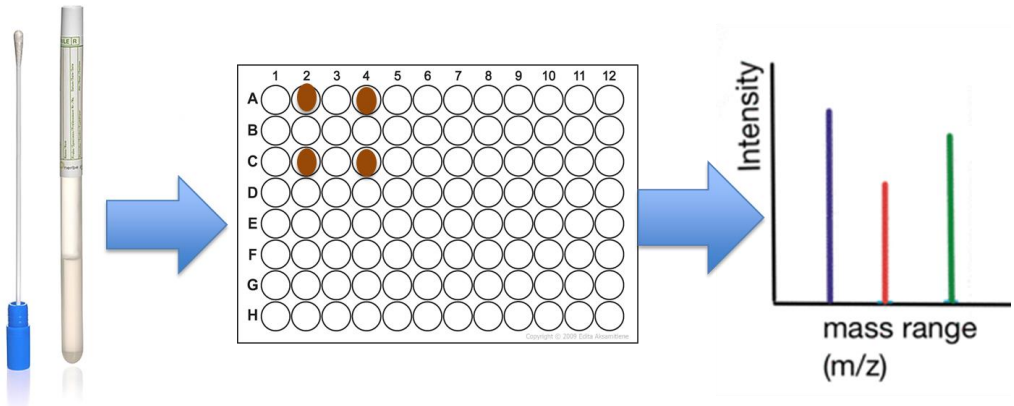


Figure 5.4. Proposed diagnostic test for rapid drug detection.

5.7. Ethanol Extraction

Ethanol can be used to extracts compounds out of sample matrixes. To determine if the use of ethanol could be beneficial to the diagnostic testing, caffeine in ear wax and ethanol was tested. Caffeine, a readily available and understood compound, was used to test the signal of analytes with and without ethanol. Caffeine standards (100ppm) in pure ethanol, pure synthetic ear wax, and diluted ear wax (1:1 ethanol ear wax mix) were tested. Figure 5.5 displays the caffeine response for each matrix and displays that ethanol increases the signal for the analyte.

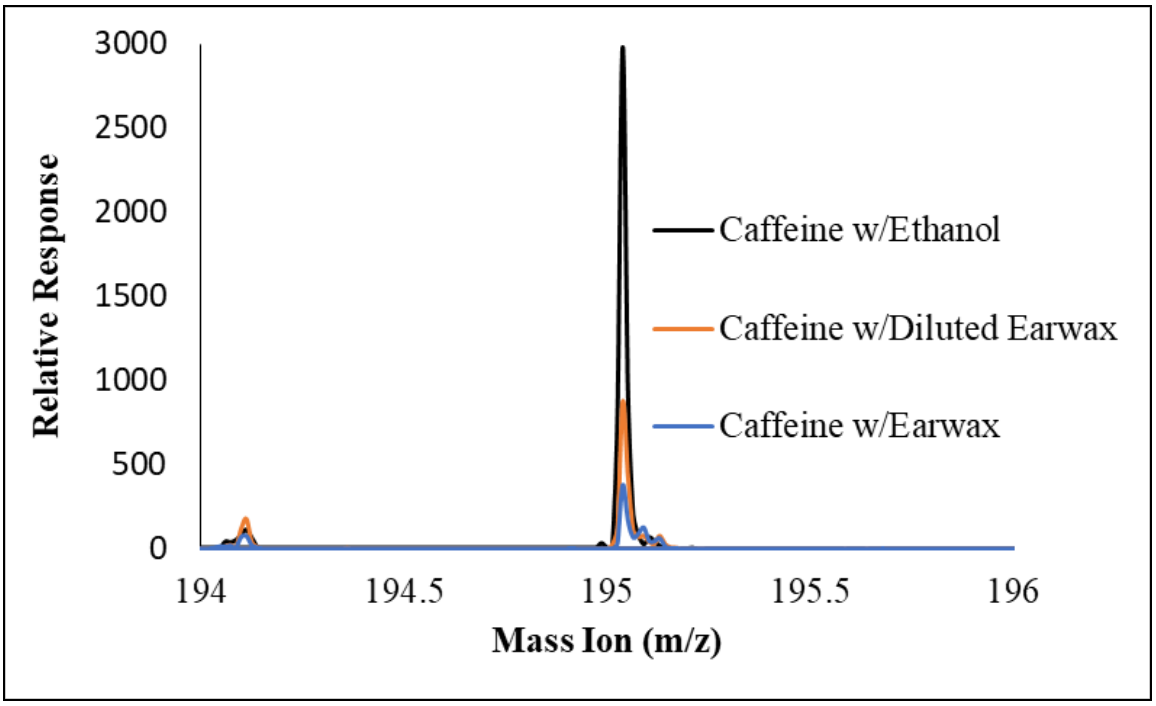


Figure 5.5. Caffeine standard comparison in ethanol (black), ethanol and ear wax mix (orange), and ear wax (blue).

5.7. Metabolites with Ethanol

Cotinine and 11-OH-THC were diluted in ethanol to explore the response and detection limits of the metabolites. Figure 5.6 and 5.7 display calibration curves for

cotinine and 11-OH-THC, respectively. The curve displays the detection limit and linearity range of the two standards dissolved in ethanol. The cotinine (Figure 5.6) had a limit of quantitation and detection of 0.4ppm and 0.1ppm, respectively. Detection limit for 11-OH-THC (Figure 5.7) was 0.01ppm and the limit of quantitation was 0.02ppm.

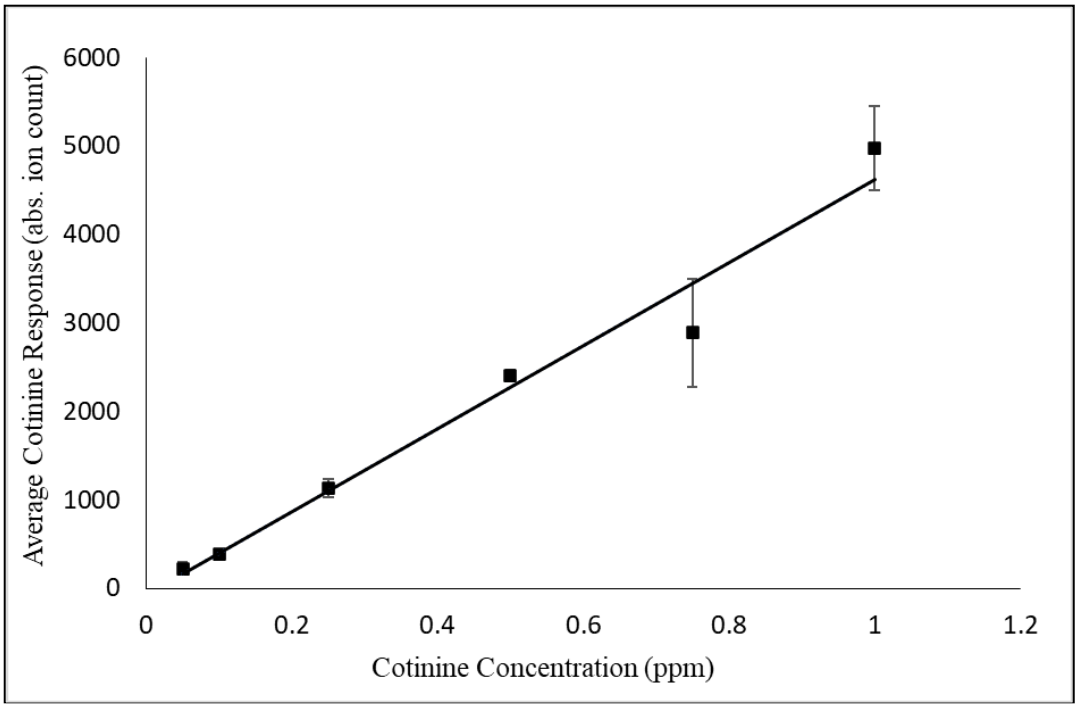


Figure 5.6. Mass intensity calibration curve for continue in ethanol ranging from 0.05 to 1ppm. The slope, intercept and R^2 values are 4697, -75.50, and 0.9720, respectively.

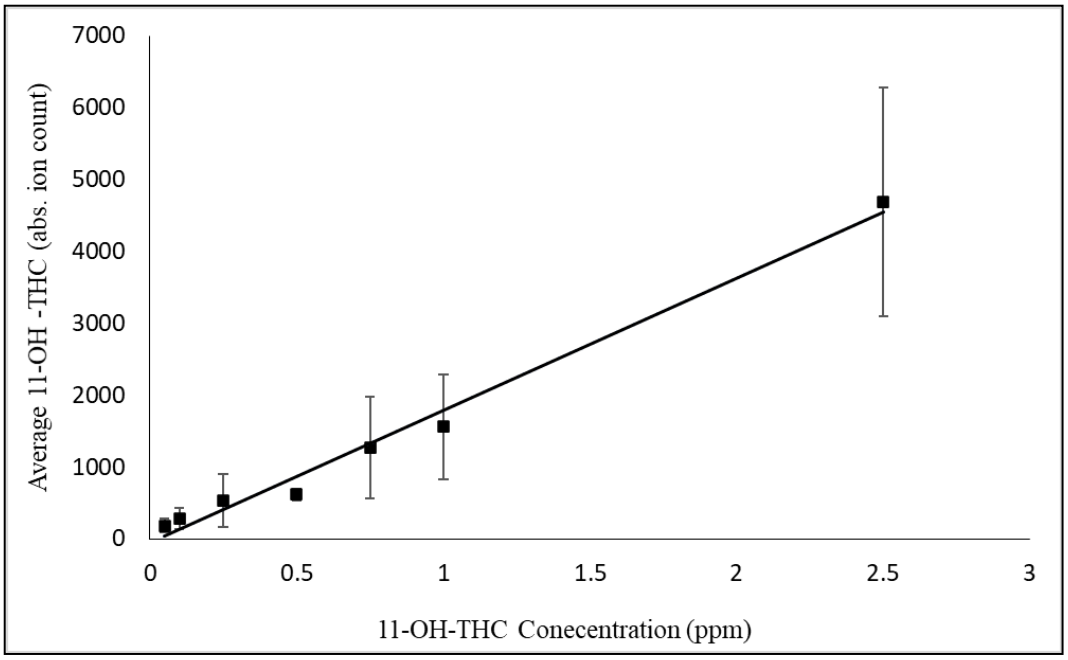


Figure 5.7. Mass intensity calibration curve for 11-OH-THC in ethanol ranging from 0.05 to 1ppm. The slope, intercept and R^2 values are 1840, -51.54, and 0.98687, respectively.

Conclusion

Cholesterol and squalene, major compounds, were detected in natural ear wax which indicated that MIDAS could be used for ear wax analysis. Synthetic ear wax was selected as a substitute for natural ear wax as a more uniform matrix and a less invasive sample collection process. Cotinine and 11-hydroxy-THC were tested both in and out of ear wax. It was determined that analytes can be detected in ear wax, but ethanol can increase signal by extraction. This allows a new diagnostic technique for rapid drug detection to be proposed. Future directions for MIDAS analysis include other secretion and biological compounds such as blood, sweat, spit, and urine for drug detection.

Chapter 6: Conclusion

Food and forensic analysis are growing fields and detection methods are needed for rapid analysis. Currently chromatographic techniques are the dominant methods used for both quantitative and qualitative analysis. These methods are very lengthy in run time and sample preparation. There are current methods being developed that utilize ambient ionization techniques which are high throughput methods. The current ambient ionization methods being used are limited by their sample plates or probes. The use of MIDAS alleviates the restriction of the sampling platform due to its ability to utilize interchangeable platforms. Testing the versatility of the of the MIDAS, applications were explored in the food and forensic field.

Stated in Chapter 2, additional modifications were made to the MIDAS to increase sensitivity and reproducibility. The removal of the glass nozzle for applications outside of TLC plate analysis increase the sensitivity and decreased the degradation and loss of sample when using the 96-well plate and glass plates. When the nozzle was removed, the pressure of the nitrogen was evaluated to determine the ideal pressure. It was determined that 80 PSI was the ideal pressure to use after the removal of the nozzle. This pressure produced the optimal signal as well as matched the same linear velocity used when the glass nozzle was utilized with a 40 PSI nitrogen pressure. The last adjustment that was made for each application was the temperature of the nitrogen gas stream. Temperature maps for each application were conducted to find the optimal setting for analysis.

In Chapter 3 vanilla products were analyzed to develop a fast characterization method and to explore potential differences in country of origin. MIDAS MS can rapidly characterize both vanilla extracts and bean fragments making MIDAS suitable for both liquid and solid samples. Vanilla compounds were detected in all pure vanilla samples with no signs of adulteration present, and imitation vanilla extracts contained the expected ethyl vanillin peak. Spiked coumarin samples were linear down to 0.01% (v/v) giving the method a limit of detection of 100ppb. A new sampling plate was designed to stabilize solid samples under a gas stream. Assaying tonka beans in addition to the spiked coumarin samples provided a method of detecting adulteration in vanilla extracts. Lastly, assaying beans from various countries showed how MIDAS can be easily used for large, irregular shaped solids that have caused a hindrance in existing ambient techniques. MIDAS shows the potential to be used in further vanilla bean analysis and could be a useful tool in distinguishing country of origin. MIDAS is internally reproducible and has some quantitative properties. MIDAS also proved to be more sensitive than existing DAPCI source, the DSA.

In Chapter 4 peppercorn were characterized to show the versatility of the MIDAS across several sample types. Major compounds for black pepper were detected in the two different peppercorns tested. The differences between ground and whole peppercorns give indication that compounds may be dispersed throughout the corns differently. V had compounds more evenly dispersed whereas B had compound more concentrated in the inside of the corn.

Papaya is one of the most common adulterants for black peppercorns due to visual

similarities. MIDAS can characterize both peppercorns and papaya seeds and easily distinguishes the differences in the spectral data between the two. Using a two-sampled Kolmogorov-Smirnov and a Z test, the differences between papaya and black peppercorns were confirmed. Spectral and statistical differences provide confirmation that the MIDAS can be used to rapidly differentiate black pepper from papaya with the aid of statistical analysis.

In Chapter 5 the exploration of drug metabolites in synthetic ear wax were explored. Major compounds were detected when a trail run of natural ear wax which gave promise that MIDAS could be used for ear wax analysis. Synthetic ear wax was selected as a substitute for natural ear wax for more uniform matrix and a less invasive sample collection process. Cotinine and 11-hydroxy-THC were tested both in and out of ear wax to determine detection limits of compounds in ear wax. It was determined that synthetic ear wax can be used as matrix for identifying forensic compounds as well as the signal of the compounds increased when the ear wax was diluted with ethanol prior to analysis. A new diagnostic technique test for a high throughput method for drug detection.

The MIDAS has the capability to be successfully utilized for various sample types including powders, beans, pastes, solid corns, and waxes. The interchangeable sample platforms and the ability to adjust the ionization angles allows MIDAS to be competitive with commercial ambient sources. MIDAS qualitative outcomes are also highly competitive with chromatographic techniques and are suggested to be more sensitive than other ambient methods.

MIDAS Proposed Modification and Future Directions:

Hardware

Since the MIDAS is an in-house built instrument there are modifications that can be made to optimize the functionality. One of the future directions that can be taken to improve the MIDAS is the addition of an enclosure around the source. Throughout the chapters it was discussed that structural modifications would be advantageous to the reproducibility of the source. If the source were to sit within an enclosed space, the humidity and temperature can be regulated. This would increase the reproducibility of the sample analysis and make the MIDAS competitive with chromatographic techniques on the quantitative scale. The enclosure would also protect the MIDAS from changes in air flow and potentially background interferences, which would increase the reproducibility while also eliminating unintended compound interaction.

Another avenue to explore is changing the capillary extender. Currently the extender is linear. The MIDAS can adjust the ionization angle to accommodate this approach, but a bend in the end of the capillary tube could increase signal. Experimenting with the angle of the bend and diameter of the open could aid in sample reducibility as well. Having the extender bent towards the plate could ensure that all the ionized sample makes it to the detector and therefore increasing sampling signal.

Currently, there are sampling plates for other ambient sources that use heat to aid in the ionization process. The next step should be a cooling plate for biomolecules. The

plate can be utilized for MIDAS analysis but would be advantageous to the entire ambient field. Using Peltier devices, a cold plate can be constructed and operated through the same power source that is attached to the corona pin. Biological samples can be rapidly ionized without the freeze/thaw process and without concern of rapid degradation during the analysis process.

Lastly, the MIDAS can be paired with any instrument that has an atmospheric inlet. Analysis with MIDAS can be compared on different instruments with varying resolutions. This could be advantageous to analyze compounds that are below the TOF's detection limits, such a benzyl isothiocyanate in papaya seeds. In addition to higher resolution instrumentation, the MIDAS is an invaluable technique to use for field analysis. Portable mass spectrometers and ion mobility spectrometers are currently used as in-field detection techniques and there are handheld DAPCI sources already created. With the MIDAS having the capability of utilizing interchangeable platforms and on a fixed platform stage, it can be an extremely useful and quick qualitative technique. Environmental and forensic fields could utilize the combination of the MIDAS with a portable detector.

Applications

A future direction for the MIDAS could be analyzing gaseous samples. The MIDAS could test gaseous samples, and this would make it competitive with headspace GC techniques. The enclosure mentioned above should be utilized for the analysis to

prevent sample leaks. Reconfiguring the sample gas line for the MIDAS should also be considered if this avenue is approached.

A forensic application that could utilize MIDAS analysis would be the detection of arson samples. This would consist of detection of accelerants on different solid sample types. Gunshot residues can be collected and assayed using different collection techniques. The adhesive plate for the MIDAS displays the opportunity of collecting residue off the weapons as well the physical body of the proposed suspect (e.g., hand). Cotton swabs can also be utilized in sample collection as well as articles of clothing. Since MIDAS analysis is considered nondestructive, clothing and paper samples would not be damaged.

There are several applications to explore with the MIDAS. The interchangeable platforms open the MIDAS up to many different possibilities. Based on preliminary data, MIDAS can be used to determine if plastics are leaching into food products. Testing both “effected” food and containers under different conditions could identify chemicals associated with plastic leaching. Testing differences in storage containers in general could be explored. When testing bottle beer and canned beer, there were differences seen in the spectra. A hypothesis is that since the can was lined with a plastic film, the compounds seen in the canned spectra may have corresponded with the film.

Another market that MIDAS could be used is detecting illicit and non-illicit drugs. The cannabis and psychedelic research market has increased significantly in the recent years. The largest problem associated with the cannabis market is that different matrixes give difficulties to current detection methods. Using MIDAS analysis could simplify cannabis testing. Currently, the analysis is split between GC and HPLC techniques because not one technique can do it all. MIDAS can detect solid, liquid, and gaseous samples it can be used to identify all compounds. Another advantage is that the physical sample does not go through the instrument, so the "sticky" nature of the cannabis products would be less of a hinderance with MIDAS analysis.

In chapter 5, the MIDAS was able to assay drug compounds that were not in viscous matrixes. This technique could be used to detect other drugs such narcotics on various surfaces. With the use of swabs or adhesive strips the MIDAS can be used for rapid drug detection. This application can be used in conjunction with a portable mass spectrometer for in field detection.

References

1. *TITLE 21--FOOD AND DRUGS CHAPTER I--FOOD AND DRUG ADMINISTRATION DEPARTMENT OF HEALTH AND HUMAN SERVICES SUBCHAPTER B - FOOD FOR HUMAN CONSUMPTION (CONTINUED) <td nowrap="">PART 189SUBSTANCES PROHIBITED FROM USE IN HUMAN FOOD*, C.o.F. Regulations, Editor. 2019, U.S. Food & Drug Administration.
2. Sasikumar, B., et al., *22 - Advances in Adulteration and Authenticity Testing of Herbs and Spices.* Advances in Food Authenticity Testing, 2016: p. 585-624.
3. Bharathi, S.K.V., et al., *Instrument-based detection methods for adulteration in spice and spice products - A review*. Journal of Spices & Aromatic Crops, 2018. **27**(2): p. 106-118.
4. Galvin-King, P., S.A. Haughey, and C.T. Elliott, *Herb and spice fraud; the drivers, challenges and detection.* Food Control, 2018. **88**: p. 85-97.
5. Osman, A.G., et al., *Overview of Analytical Tools for the Identification of Adulterants in Commonly Traded Herbs and Spices.* Journal of AOAC International, 2019. **102**(2): p. 376-385.
6. Hong, E., et al., *Modern analytical methods for the detection of food fraud and adulteration by food category.* Journal of the Science of Food and Agriculture, 2017. **97**(12): p. 3877-3896.
7. de Jager, L.S., G.A. Perfetti, and G.W. Diachenko, *Determination of coumarin, vanillin, and ethyl vanillin in vanilla extract products: liquid chromatography mass spectrometry method development and validation studies.* Journal of Chromatography A, 2007. **1145**(1): p. 83-88.
8. Lavine, B.K., D.T. Corona, and U.D.N.T. Perera, *Analysis of vanilla extract by reversed phase liquid chromatography using water rich mobile phases.* Microchemical Journal, 2012. **103**: p. 49-61.
9. Lee, P.J., et al., *Analysis of vanilla flavoured food products using atmospheric-pressure solids analysis probe*. 2010, TEKNOSCIENZE S.R.L.: Italy. p. 25.
10. Inai, Y., et al., *Identification of the Key Odorants in Tahitian Cured Vanilla Beans (Vanilla tahitensis) by GC-MS and an Aroma Extract Dilution Analysis AU - TAKAHASHI, Makoto.* Bioscience, Biotechnology, and Biochemistry, 2013. **77**(3): p. 601-605.
11. Dalmia, A., G.L. Perkins, and C. Whitehouse, *Rapid Differentiation Between Natural and Artificial Vanilla Flavorings for Determining Food Fraud Using AxION DSA/TOF with No Chromatography or Sample Preparation.* PerkinElmer, Inc. Application Note, 2013.
12. Santos, I.C., J. Smuts, and K.A. Schug, *Rapid Profiling and Authentication of Vanilla Extracts Using Gas Chromatography-Vacuum Ultraviolet Spectroscopy.* Food Analytical Methods, 2017. **10**(12): p. 4068-4078.
13. Takahashi, M., S. Sakamaki, and A. Fujita, *Simultaneous Analysis of Guaiacol and Vanillin in a Vanilla Extract by Using High-Performance*

Liquid Chromatography with Electrochemical Detection. Bioscience, Biotechnology, and Biochemistry, 2013. **77**(3): p. 595-600.
14. Chadwick, A., *Natural disaster, other factors pushing up vanilla prices.* Winston-Salem Journal, 2018.
15. Kaunzinger, A., D. Juchelka, and A. Mosandl, *Progress in the Authenticity Assessment of Vanilla. 1. Initiation of Authenticity Profiles.* Journal of Agricultural and Food Chemistry, 1997. **45**(5): p. 1752-1757.
16. Dalmia, A., *Detection of Vanilla Bean Extract Adulteration with Tonka Bean Extract with No Chromatography or Sample Preparation.* PerkinElmer, Inc. Application Note, 2013.
17. Takahashi, M., et al., *Identification of the Key Odorants in Tahitian Cured Vanilla Beans (Vanilla tahitensis) by GC-MS and an Aroma Extract Dilution Analysis.* Bioscience, Biotechnology, and Biochemistry, 2013. **77**(3): p. 601-605.
18. Marles, R., C. Compadre, and N. R. Farnsworth, *Coumarin in vanilla extracts: Its detection and significance*. Vol. 41. 1987. 41-47.
19. Garcia, O., C. Keeney, and S. Coticone, *Detection of Coumarin in Artificially Adulterated Vanilla Bean Extracts in a Forensic Science Laboratory*. Vol. 20. 2015.
20. Chandra, P., et al., *Metabolic profiling of Piper species by direct analysis using real time mass spectrometry combined with principal component analysis.* Analytical Methods, 2014. **6**(12): p. 4234-4239.
21. Ahmad, A., et al., *Quantification of total phenol, flavonoid content and pharmacognostical evaluation including HPTLC fingerprinting for the standardization of Piper nigrum Linn fruits.* Asian Pacific Journal of Tropical Biomedicine, 2015. **5**(2): p. 101-107.
22. Bansal, S., et al., *Food adulteration: Sources, health risks, and detection methods.* Critical Reviews in Food Science and Nutrition, 2017. **57**(6): p. 1174-1189.
23. Medina, S., et al., *Food fingerprints – A valuable tool to monitor food authenticity and safety.* Food Chemistry, 2019. **278**: p. 144-162.
24. R. Graham Cooks, a., et al., *Ambient Mass Spectrometry.* Science, 2006(5767): p. 1566.
25. Dalmia, A., *Rapid Measurement of Food Adulteration with Minimal Sample Preparation and No Chromatography Using Ambient Ionization Mass Spectrometry.* Journal of AOAC International, 2017. **100**(2): p. 573-575.
26. Pavlovich, M.J., E.E. Dunn, and A.B. Hall, *Chemometric brand differentiation of commercial spices using direct analysis in real time mass spectrometry.* Rapid Communications in Mass Spectrometry, 2016. **30**(9): p. 1123-1130.
27. Moore, J.C., J. Spink, and M. Lipp, *Development and Application of a Database of Food Ingredient Fraud and Economically Motivated Adulteration from 1980 to 2010.* Journal of Food Science, 2012. **77**(4): p. R118-R126.
28. Friedman, M., et al., *Analysis by HPLC and LC/MS of Pungent Piperamides in Commercial Black, White, Green, and Red Whole and Ground*

Peppercorns. Journal of Agricultural and Food Chemistry, 2008. **56**(9): p. 3028-3036.
29. Jirovetz, L., et al., *Aroma compound analysis of Piper nigrum and Piper guineense essential oils from Cameroon using solid-phase microextraction–gas chromatography, solid-phase microextraction–gas chromatography–mass spectrometry and olfactometry.* Journal of Chromatography A, 2002. **976**(1): p. 265-275.
30. Curl, C.L. and G.R. Fenwick, *On the determination of papaya seed adulteration of black pepper*. Food Chemistry, 1983. **12**(4): p. 241-247.
31. Bajpai, V., et al., *Profiling of Piper betle Linn. cultivars by direct analysis in real time mass spectrometric technique.* Biomedical Chromatography, 2010. **24**(12): p. 1283-1286.
32. Gross, J., *Direct analysis in real time-a critical review on DART-MS.* Analytical & Bioanalytical Chemistry, 2014. **406**(1): p. 63-80.
33. Winter, G.T., *Development of the Molecular Ionization Desorption Analysis Source (midas) for Mass Spectrometry*. 2016: University of Maryland, Baltimore County.
34. Winter, G.T., J.A. Wilhide, and W.R. LaCourse, *Molecular Ionization-Desorption Analysis Source (MIDAS) for Mass Spectrometry: Thin-Layer Chromatography.* Journal of The American Society for Mass Spectrometry, 2016. **27**(2): p. 352-358.
35. Venter, A.R., et al., *Mechanisms of Real-Time, Proximal Sample Processing during Ambient Ionization Mass Spectrometry.* Analytical Chemistry, 2014. **86**(1): p. 233-249.
36. Thomson, G.W., *The Antoine Equation for Vapor-pressure Data.* Chemical Reviews, 1946. **38**(1): p. 1-39.
37. Pitman, C.N. and W.R. LaCourse, *Desorption atmospheric pressure chemical ionization: A review.* Analytica Chimica Acta, 2020. **1130**: p. 146-154.
38. Yang, S., et al., *Detection of Melamine in Milk Products by Surface Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry.* Analytical Chemistry, 2009. **81**(7): p. 2426-2436.
39. Yang, S.-P., et al., *Imaging Melamine in Egg Samples by Surface Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry.* Chinese Journal of Analytical Chemistry, 2009. **37**(3): p. 315-318.
40. Williams, J.P., et al., *The use of recently described ionisation techniques for the rapid analysis of some common drugs and samples of biological origin.* Rapid Communications in Mass Spectrometry, 2006. **20**(9): p. 1447-1456.
41. Williams Jonathan, P. and H. Scrivens James, *Rapid accurate mass desorption electrospray ionisation tandem mass spectrometry of pharmaceutical samples.* Rapid Communications in Mass Spectrometry, 2005. **19**(24): p. 3643-3650.
42. Chen, H.-W., et al., *Instrumentation and Characterization of Surface Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry.* Chinese Journal of Analytical Chemistry, 2007. **35**(8): p. 1233-1240.
43. Chen, H., et al., *Surface desorption atmospheric pressure chemical ionization mass spectrometry for direct ambient sample analysis without toxic chemical contamination.* Journal of Mass Spectrometry, 2007. **42**(8): p. 1045-1056.

44. Wu, Z., et al., *Differentiation of Dried Sea Cucumber Products from Different Geographical Areas by Surface Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry.* Journal of Agricultural and Food Chemistry, 2009. **57**(20): p. 9356-9364.
45. Zhang, X., et al., *Tracing Origins of Complex Pharmaceutical Preparations Using Surface Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry.* Analytical Chemistry, 2010. **82**(19): p. 8060-8070.
46. Good, A., D.A. Durden, and P. Kebarle, *Ion–Molecule Reactions in Pure Nitrogen and Nitrogen Containing Traces of Water at Total Pressures 0.5–4 torr. Kinetics of Clustering Reactions Forming H+(H2O)n.* The Journal of Chemical Physics, 1970. **52**(1): p. 212-221.
47. Horning, E.C., et al., *New picogram detection system based on a mass spectrometer with an external ionization source at atmospheric pressure.* Analytical Chemistry, 1973. **45**(6): p. 936-943.
48. Dzidic, I., et al., *Comparison of positive ions formed in nickel-63 and corona discharge ion sources using nitrogen, argon, isobutane, ammonia and nitric oxide as reagents in atmospheric pressure ionization mass spectrometry.* Analytical Chemistry, 1976. **48**(12): p. 1763-1768.
49. Andrade, F.J., et al., *Atmospheric Pressure Chemical Ionization Source. 1. Ionization of Compounds in the Gas Phase.* Analytical Chemistry, 2008. **80**(8): p. 2646-2653.
50. *Mass Analyzer - Time of Flight.*
51. Botch-Jones, S., et al. *The detection of NBOMe designer drugs on blotter paper by high resolution time-of-flight mass spectrometry (TOFMS) with and without chromatography*. 2016. Switzerland: Elsevier Science B.V., Amsterdam.
52. Hazleton, L.W., et al., *Toxicity of coumarin.* The Journal of pharmacology and experimental therapeutics, 1956. **118**(3): p. 348-358.
53. European Food Safety, A., *Coumarin in flavourings and other food ingredients with flavouring properties - Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC).* EFSA Journal, 2008. **6**(10): p. 793.
54. Loncar, M., et al., *Coumarins in Food and Methods of Their Determination*. 2020.
55. *Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC*. 2008, OFFICE FOR OFFICIAL PUBLICATIONS OF THE EEC: Great Britain. p. 34.
56. Vijayalakshmi. S, D.X.C.S., *Vanilla- Natural Vs Artificial: A Review*. 2019.
57. *Vanilla composition and vanillin content*. KAU Agri-Infotech Portal 2019 [cited 2019 July]; Available from: http://www.celkau.in/crops/spices/Vanilla/vanilla_composition_and_vanillin_content.aspx.

58. Rao, S.R. and G.A. Ravishankar, *Vanilla flavour: production by conventional and biotechnological routes*. 2000, ELSEVIER APPLIED SCIENCE: Great Britain. p. 289.
59. Sharp, M.D., et al., *Rapid Discrimination and Characterization of Vanilla Bean Extracts by Attenuated Total Reflection Infrared Spectroscopy and Selected Ion Flow Tube Mass Spectrometry.* Journal of Food Science, 2012. **77**(3): p. C284-C292.
60. Belay, M.T. and C.F. Poole, *Determination of vanillin and related flavor compounds in natural vanilla extracts and vanilla-flavored foods by thin layer chromatography and automated multiple development.* Chromatographia, 1993. **37**(7): p. 365-373.
61. Jjunju, F.P.M., et al., *Hand-Held Portable Desorption Atmospheric Pressure Chemical Ionization Ion Source for in Situ Analysis of Nitroaromatic Explosives.* Analytical Chemistry, 2015. **87**(19): p. 10047-10055.
62. Virkler, K. and I.K. Lednev, *Analysis of body fluids for forensic purposes: From laboratory testing to non-destructive rapid confirmatory identification at a crime scene.* Forensic Science International, 2009. **188**(1): p. 1-17.
63. Koster, R.A., et al., *Fast and highly selective LC-MS/MS screening for THC and 16 other abused drugs and metabolites in human hair to monitor patients for drug abuse.* Ther Drug Monit, 2014. **36**(2): p. 234-43.
64. Meier, S.I., et al., *Analysis of drugs of abuse in Cerumen - correlation of postmortem analysis results with those for blood, urine and hair.* Drug Testing and Analysis, 2017. **9**(10): p. 1572-1585.
65. Shokry, E., et al., *Earwax as an alternative specimen for forensic analysis.* Forensic Toxicology, 2017. **35**(2): p. 348-358.
66. Shokry, E. and N.R.A. Filho, *Insights into cerumen and application in diagnostics: past, present and future prospective.* Biochemia medica, 2017. **27**(3): p. 030503-030503.
67. Vindenes, V., et al., *Detection of drugs of abuse in simultaneously collected oral fluid, urine and blood from Norwegian drug drivers.* Forensic Science International, 2012. **219**(1): p. 165-171.
68. Shokry, E., et al., *Earwax: an innovative tool for assessment of tobacco use or exposure. A pilot study in young adults.* Forensic Toxicology, 2017. **35**(2): p. 389-398.
69. Guest, J.F., et al., *Impacted cerumen: composition, production, epidemiology and management.* Qjm, 2004. **97**(8): p. 477-88.
70. Okuda, I., et al., *The organic composition of earwax.* J Otolaryngol, 1991. **20**(3): p. 212-5.
71. Nicolaou, A.G., et al., *Application of an ultra-performance liquid chromatography-tandem mass spectrometric method for the detection and quantification of cannabis in cerumen samples.* Journal of Chromatography A, 2021. **1642**: p. 462035.
72. Kumar, P., et al., *Use of Spectroscopic Methods and Their Clinical Applications in Drug Abuse: A Review.* Critical Reviews in Analytical Chemistry, 2021: p. 1-14.

73. Barbosa, J.M.G., et al., *Cerumenogram: a new frontier in cancer diagnosis in humans.* Scientific Reports, 2019. **9**(1): p. 11722.
74. Herane-Vives, A., et al., *Measuring Earwax Cortisol Concentration using a non-stressful sampling method.* Heliyon, 2020. **6**(11): p. e05124.