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Thesis

DIFFERENT EXTRACTION EFFICIENCIES OBSERVED FROM SYNTHETIC CANNABINOID ANALYSIS DUE TO BURNING AND MATRIX EFFECTS

by

HEATHER GRAY MOWATT

B.S., Queen's University, 2009

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

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Approved by

First Reader ____

Adam B. Hall, Ph.D., D-ABC
Instructor, Program in Biomedical Forensic Sciences
Boston University School of Medicine

Second Reader ____

Richard Laing, M.S.
Manager, Drug Analysis Services Vancouver Laboratory
Health Canada

Dedications

This thesis is dedicated to Dr. Scott Gray and Patricia Mowatt, whose love and passion for learning continue to motivate and inspire me every day.

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DIFFERENT EXTRACTION EFFICIENCIES OBSERVED FROM SYNTHETIC CANNABINOID ANALYSIS DUE TO BURNING AND MATRIX EFFECTS

HEATHER GRAY MOWATT

Boston University School of Medicine, 2013

Major Advisor: Adam B. Hall, Ph.D., Instructor, Program in Biomedical Forensic Sciences

ABSTRACT

Cannabinoids are compounds that are naturally present in *Cannabis sativa L.*, which interact with cannabinoid receptors in the nervous system, known as CB1 and CB2 receptors. The most abundant and well-known cannabinoid that can be isolated from cannabis is Δ^9-tetrahydrocannabinol (THC). The structure of this compound specifically allows interaction with the CB1 and CB2 receptors, known as cannabimimetic activity. Other compounds have since been produced, inspired by THC, which have been designed to elicit similar pharmacological responses, and therefore are beneficial as analgesics. These compounds are known as synthetic cannabinoids.

Synthetic cannabinoids, while potentially useful as therapeutic treatments for pain, are currently also popular as recreational drugs. Herbal products that contain synthetic cannabinoids are sold as "legal highs," as few of these compounds are illegal according to the Controlled Drugs and Substances Act. These products are prepared by combining synthetic cannabinoids and plant material, and are smoked similar to marijuana. As the legality of many synthetic cannabinoids is quickly decreasing, as evidenced by the March 2011 emergency scheduling of five such compounds, it is becoming increasingly likely that these products will soon become popular exhibits to be submitted to controlled substances laboratories for testing. If a previously smoked product is submitted, there could potentially be effects due to the burning, the presence of the plant or paper substrate, and other synthetic cannabinoids that could directly diminish the facility of analysis. The aim of this thesis was to investigate these effects using four synthetic cannabinoids (AM-2201, JWH-015, HU-211, and RCS-4) and four substrates (tobacco, rolling paper, mint, and rosemary).

Results demonstrated diminished peak areas, which are likely due to the introduction of these variables, which include burning the drug of abuse, and spiking the drug of abuse onto various matrices. The trend of lower peak areas

further suggests that burning, the presence of plant material, and other cannabinoids potentially all compromise the facility of analyzing synthetic cannabinoid products. The act of burning one synthetic cannabinoid in particular, AM-2201, appeared to greatly decrease the capability to detect the analyte, as did the application of AM-2201 to various substrates. Furthermore, the ability to detect AM-2201 appeared to vary greatly between results obtained from analyzing samples applied to different substrates. Analysis of cannabinoid mixtures demonstrated that GC/MS analysis of different cannabinoids gave various peak areas although the concentrations remained consistent. Peak area ratios of cannabinoid mixtures that were extracted from substrates were found to not differ significantly between the specific substrates studied. This research supports that all of these variables should therefore be considered in regards to analysis of herbal products containing synthetic cannabinoids.

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List of Abbreviations

AAPCC	American Association of Poison Control Center
AM	Alexandros Makriyannis
AM-2201	1-(5-fluoropentyl)-3-(1-naphthoyl) indole
CBD	Cannabidiol
CBN	Cannabinol
CNS	Central Nervous System
DEA	Drug Enforcement Administration
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
GC/MS	Gas Chromatography/Mass Spectrometry
HU	Hebrew University
HU-210	3-(1,1'-dimethylheptyl)-6aR,7,10,10aR-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol
HU-211	3-(1,1-dimethylheptyl)-6aS,7,10,10aS-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol
JWH	John W. Huffman

JWH-015	(2-methyl-1-propyl-1H-indol-3-yl)-1-naphthalenyl methanone
JWH-018	(1-pentyl-1H-indol-3-yl)-1-naphthalenyl methanone
LC	Liquid Chromatography
LC/MS	Liquid Chromatography/Mass Spectrometry
LLE	Liquid-Liquid Extraction
m/z	Mass-to-Charge
MDMA	3,4-methylenedioxy-*N*-methamphetamine
mL	Milliliter
NCC	Non-classical Cannabinoids
PNS	Peripheral Nervous System
RCS-4	(4-methoxyphenyl)(1-pentyl-1H-indol-3-yl) methanone
SIM	Selective Ion Monitoring
SWGDRUG	Scientific Working Group for the Analysis of Seized Drugs
THC	Δ^9-tetrahydrocannabinol
TLC	Thin-Layer Chromatography
ug	Microgram

I. Introduction:

1. Cannabis and Cannabinoids

1.1 Popularity of Cannabis products

Narcotic agents have been abused throughout history, and the number of drugs that are commonly abused is consistently increasing. The constant demand for new drugs of abuse leads to the development of new products, which are frequently regulated by the Drug Enforcement Administration (DEA). For example, 3,4-methylenedioxy-*N*-methamphetamine (MDMA) was developed during the 1980s era, but became the most popular during the 1990s, and was not classified as Schedule I on the Controlled Drugs and Substances Act until 2003. By 2003, other products had emerged on the market, which caused a decrease in the relative popularity of MDMA.[1] The trend of MDMA popularity, therefore, followed a pattern that is often observed with many drugs of abuse: development, followed by increased popularity, subsequent regulation, and eventually a decrease in popularity compared to other drugs. There are, however, particular controlled substances that remain prevalent in commercial markets in relation to both demand and supply. Cannabis products and Δ^9-tetrahydrocannabinol (THC) products remain the most ubiquitous and the most frequently encountered substances in the United States. In 2010, approximately

587,399 samples of the 1,713,360 drug exhibits that were submitted to forensic laboratories for analysis (approximately 34%) were determined to be cannabis or THC products.[2]

1.2 Analysis of Cannabis and Components

Cannabis submissions typically consist of plants (*Cannabis sativa L.* and related members of Cannabinaceae family) and resins or oils that are extracted from plant material.[3] Cannabis sativa L. (cannabis), or marijuana samples can be presumptively identified in forensic laboratories by the characteristic cystolithic hairs and a positive Duquenois Levine test, while a conclusive identity of cannabis relies on the presence of specific naturally occurring compounds known as cannabinoids.

Cannabinoids are defined as the C_{21} compounds contained within cannabis.[3]. The most abundant cannabinoid present in cannabis was determined to be Δ^9-tetrahydrocannabinol (THC), which was isolated in 1964[4]. Thus, the chemical definition of cannabinoids expanded to specifically encompass THC and respective analogues of THC.[3] This definition can be further expanded to include unique pharmacological actions, referred to as cannabimimetic activity[5]. Cannabimimetic activity specifically describes the activation of certain receptors known as CB1 and CB2 receptors[5]. CB1 receptors are present in the central

nervous system (CNS) and CB2 receptors are present in the peripheral nervous system (PNS).[5] Although other cannabinoids present in cannabis may elicit cannabimimetic activity, the overall cannabimimetic activity of cannabis is defined by that of THC in particular. In essence, the cannabimimetic activity exhibited by cannabis is determined exclusively by that of THC. A possible reason for the unique mechanism of THC-related cannabimimetic activity is likely due to its structure. The cannabimimetic activity associated with THC has been previously connected with three structural regions on the THC molecule (Figure 1) that specifically activate the CB1 receptor. These pertinent groups include a characteristic cyclohexene ring, a hydroxyl functional group, and a carbon chain.

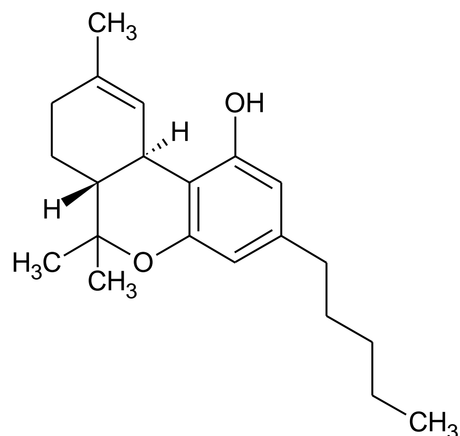


Figure 1. Structure of Δ^9-tetrahydrocannabinol (THC)

In compounds that are structurally similar to THC, the amount of CB1 activity has been directly correlated to the length of the carbon chain bonded to the phenol.[6] Although the structures of other natural cannabinoids present in cannabis, such as cannabidiol (CBD) and cannabinol (CBN), also feature these groups, the cannabimimetic activity displayed by cannabis is primarily attributed to THC. Due to a lower affinity for the CB1 and CB2 receptors compared to THC,[41] the activity of CBD is instead categorized by the different pharmacological responses observed from other receptors that are not linked to the psychotropic effects of cannabis.[7]

2. Development of Synthetic Cannabinoids

Activation of the cannabinoid receptors has been connected to the alleviation of inflammation and pain, which has caused CB1 and CB2 agonists to be subjects of high interest for researching possible analgesics.[8] As THC is a well-known agonist of both CB1 and CB2 receptors, compounds began to be developed that were structurally similar to THC while maintaining unique structural properties; these were then identified as synthetic cannabinoids. Synthetic cannabinoids began to be developed as potential therapeutic agents based on similar cannabimimetic activities to THC, therefore presenting the potential for use as analgesic treatments.[5] Pfizer pioneered this effort by

producing various compounds, known as non-classical cannabinoids (NCC's) in attempt to discover drugs that would mimic the sedative and analgesic effects of cannabis without the damaging adverse effects. One of the more notable NCC's developed by Pfizer was CP 55,940. Indeed, CP 55,940 was particularly relevant to the study of synthetic cannabinoids as the associated research led to the discovery of the CB1 and CB2 receptors.[9] The structure of CP 55,940 (Figure 2) features similar groups to THC, which subsequently causes its cannabimimetic activity to be high. The cannabimimetic activity of CP 55,940 is increased relative to THC due to the presence of unique functional groups. Consequently, groups such as longer carbon chains also cause the potency of CP 55,940 to be higher than that of THC. Specifically, the hydroxypropyl chain increases the cannabimimetic activity, relative to other Pfizer-produced CP compounds like CP 47,947, by a factor of 20. The potency of CP 55,940 is therefore increased to be approximately 100 times higher than THC.[9]

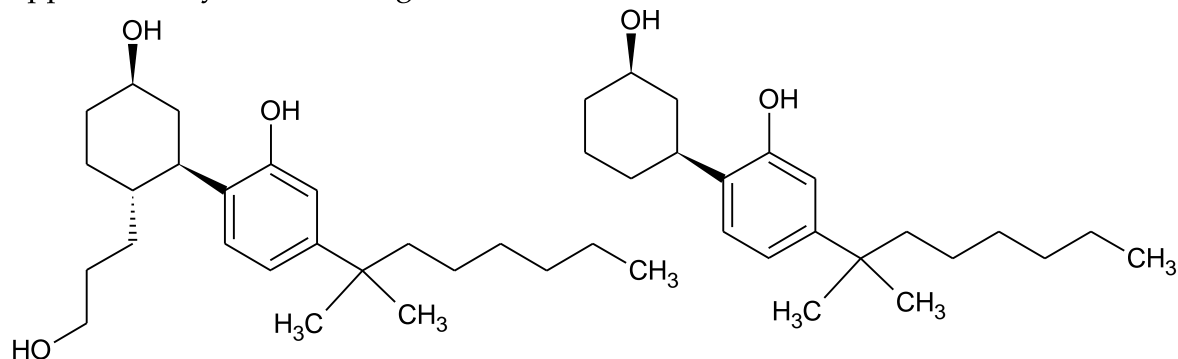


Figure 2. Structures of CP 55,940 (left) and CP 47,947 (right)

Many research groups have continued the work of Pfizer, striving to discover and understand the pharmacological properties, and potential benefits, of synthetic cannabinoids. Consequently, a high number of new synthetic cannabinoids have been developed. The newly developed compounds all exhibit different structural characteristics, which results in varying levels of cannabimimetic activity.

An example of the more prominent research groups currently working on projects related to synthetic cannabinoids includes the John W. Huffman group, who has developed hundreds of synthetic cannabinoid compounds known as JWH cannabinoids.[10] The aim of many of Huffman's studies is to create simplified compounds that elicit typical cannabinoid responses. The group therefore focuses on certain structural characteristics that can be altered and the changes in cannabimimetic activity that those changes incur. Using the conclusions made by the Winthrop group (responsible for the WIN class of synthetic cannabinoids), namely that indole derivatives also demonstrate evidence of binding to cannabinoid receptors, the Huffman group strives to determine key aspects of structures that could either amplify or diminish cannabimimetic activity at either the CB1 or CB2 receptors. One of their more relevant findings was the discovery that aminoalkyl groups bonded to indole

nitrogens, believed by the Winthrop group to be necessary for cannabimimetic activity, could be substituted by simpler alkyl groups. Additionally, by manipulating the number of carbons present in this alkyl chain, the relative affinities of the resulting compounds can be controlled, as seen with the pentyl-containing JWH-007 (which demonstrates high affinity for CB1 receptors and lower affinity for CB2 receptors) and the propyl-containing JWH-015 (which demonstrates low affinity for CB1 receptors and higher affinity for CB2 receptors).[40] The structures of JWH-007 and JWH-015 are displayed in Figure 3.

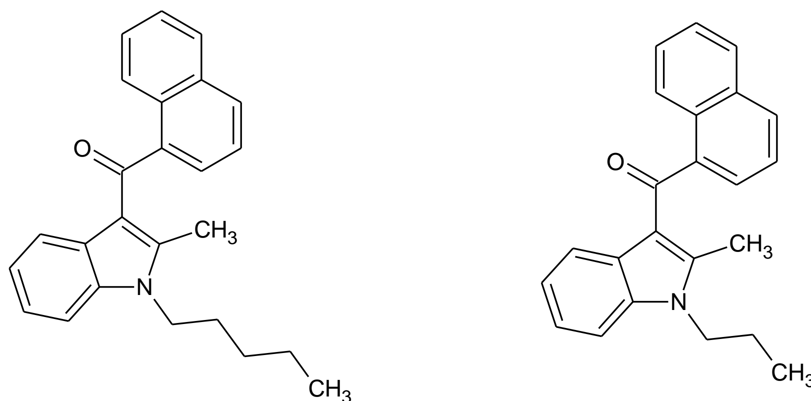


Figure 3. Structures of JWH-007 (left) and JWH-015 (right)

Further work in the field of synthetic cannabinoids and how differences in structure can lead to differences in cannabimimetic activity has been explored by the research groups at The Hebrew University in Jerusalem, where the HU class of synthetic cannabinoids has been developed.[11] The Mechoulam group synthesized HU-210 and its enantiomer, HU-211, and observed that while HU-

210 appeared to show high cannabimimetic activity (approximately 100 times higher than THC), the other was effectively inert to cannabinoid receptors and was considered inactive (HU-211).[12]

Another significant aspect to the growing field of synthetic cannabinoid research is the investigation of and selectively activating one cannabinoid receptor over another. This area of research is one pursued by the Alexandros Makriyannis group. The classes of compounds currently being developed by this group, known as AM cannabinoids, consist of those specifically designed to activate the CB2 receptor in attempt to alleviate neuropathic pain. These compounds, however, demonstrate no interaction with the CB1 receptor.[13]

2.1 Use and Observed Social Trends of Synthetic Cannabinoids

Despite abundant research focused on the development of synthetic cannabinoids for use in pharmaceuticals, the compounds are commonly used as new drugs of abuse. Cannabis products continue to be commonly used, but recent popular demand appears to have shifted to include synthetic cannabinoids. Products that contain synthetic cannabinoids appear to be the illicit substances of choice, especially among the youth, and the market has responded accordingly as evidenced by the release of highly variable and available commercial products that have been sprayed or spiked with synthetic

cannabinoids.[14] The most famous of these products include the popular "K2" and "spice" mixtures[15] though other examples such as "Hayze Hurricane", "Charge", and "Hayze trainwreck" are also sold.[16] These herbal products became available in 2006, and have since been sold as "legal highs."[17]

The increased popularity of synthetic cannabinoid products due to their current legality and presumed benign nature is concerning because of the resulting health problems related to their use.[18] Symptoms that have been reported to be correlated to synthetic cannabinoid use include tachycardia, hypertension, chest pain, heart palpitations, hallucinations, racing thoughts, and seizures.[19] In addition to the reports of these symptoms, evidence of potential harm due to synthetic cannabinoid use has also been recently explored and presented. For example, the number of calls to the American Association of Poison Control Center (AAPCC) for cases connected to K2 smoking from 2009 to 2010 increased from 53 to more than 2500.[16]

In response to the reports of increasing abuse of herbal products, the DEA declared five commonly encountered synthetic cannabinoids as Schedule I on the Controlled Drugs and Substances Act in March 2011.[20] The scheduling of the five most commonly encountered cannabinoids also aimed to deter the abuse of cannabinoid drugs that were believed to be responsible for serious medical

conditions. These compounds included (1-pentyl-1H-indol-3-yl)-1-naphthalenyl methanone (JWH-018), (1-butyl-1H-indol-3-yl)-1-naphthalenyl-methanone (JWH-073), [1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl]-1-naphthalenyl methanone (JWH-200), 2-[(1R,3S)-3-hydroxycyclohexyl]- 5-(2,methyloctan-2yl)phenol (CP-47,497), and 2-[(1R,3S)-3-hydroxycyclohexyl]- 5-(2-methylnonan-2-yl) phenol (cannabicyclohexanol). The structures of these compounds are shown in Figure 4.

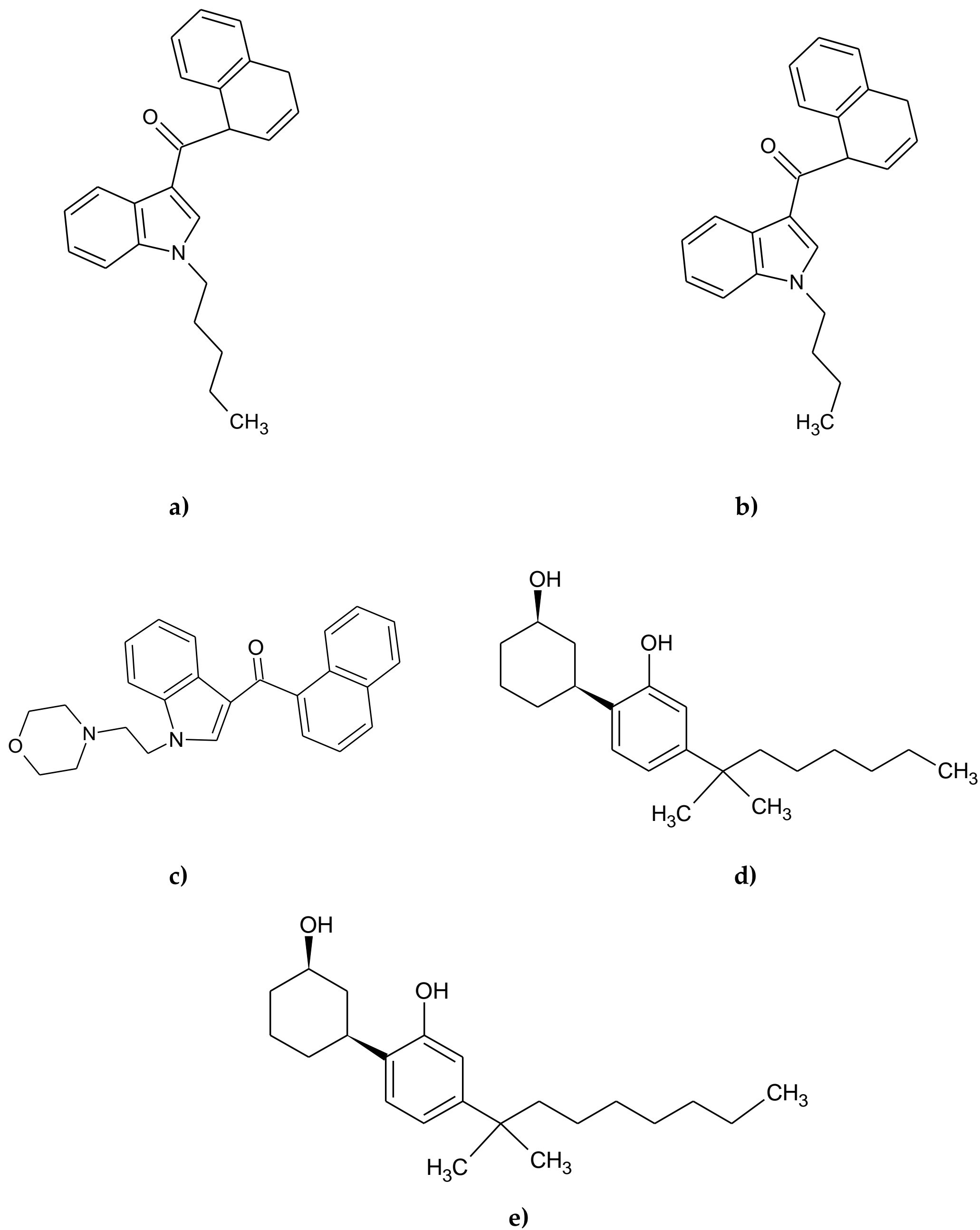


Figure 4. Structures of synthetic cannabinoids that were categorized as Schedule I in March 2011 due to DEA emergency ban: a) JWH-018, b) JWH-073, c) JWH-200, d) CP-47,497, and e) Cannabicyclohexanol.

2.2 Current Research of Synthetic Cannabinoids

The increasing demand for synthetic cannabinoids and herbal incense products has directly influenced the research performed, which focuses on the components in specific herbal incense products. Consequently, a more detailed understanding of the relative popularity of the different synthetic cannabinoids has been obtained.[7,17,19,21,22,23,25] Similar research has furthermore exposed the global phenomenon of synthetic cannabinoid distribution, as the products are sold on an international scale through the utilization of the internet.[29,31,34,35] Another important benefit of synthetic cannabinoid research is the creation of databases and spectral libraries for synthetic cannabinoids. Both the extensive study of various products, and the continuous discovery of new synthetic cannabinoids, can lead to valuable information. Specifically, this consists of more detailed knowledge of chemical properties of synthetic cannabinoids that are encountered, and more knowledge in relation to the common synthetic cannabinoids observed to be in individual herbal products, all of which could contribute to the formation of databases. Commercial products could be organized based on the synthetic cannabinoids they contain, and specific synthetic cannabinoids could potentially be targeted for analysis if knowledge of

a given brand of herbal product can give insight into which specific cannabinoids are commonly present in that brand.

Similarly, the range of methods used to analyze synthetic cannabinoids is also an area of increasing interest for research. Toxicology studies that focus on synthetic cannabinoid extraction and analysis feature the utilization of solid phase extraction and GC/MS to analyze cannabinoids contained in plasma.[26] The synthetic cannabinoid targeted by the Batista group was ajulemic acid (AJA), a non-psychoactive synthetic cannabinoid derived from 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), a metabolite of THC. For this study, GC/MS was successfully used to determine concentrations of AJA as low as 10ng/mL. GC/MS has also been expanded to include two-dimensional gas chromatography systems to analyze synthetic cannabinoids in oral fluid.[7] Liquid chromatography methods are also being developed by groups such as the Auwarter group, who is working toward optimizing a procedure of using liquid-liquid extraction (LLE) to extract synthetic cannabinoids present in serum, followed by using a liquid chromatography (LC) system in tandem with mass spectrometry.[29]

While research is currently being conducted in order to improve the detection of synthetic cannabinoids, there are also studies aimed to associate

synthetic cannabinoid use with observable medical symptoms. The investigation of various conditions that are reportedly connected with synthetic cannabinoid use leads to generation of social data about the popularity of synthetic cannabinoids. Most notably, the trend of synthetic cannabinoid use among teenagers has been intensely studied in attempt to further understand the relative popularities of the various synthetic cannabinoids[14,15,16] and also to glean the specific symptoms that can be attributed to synthetic cannabinoid use.[18] Common symptoms that were observed included both physical and psychoactive effects, including palpitations, tremors, changes in appetite, and blackouts, as well as feelings of euphoria, anxiety, changes in perception, and irritability.

3. Objective

One difference between synthetic cannabinoids and other products that are similarly abused is their preparation. Exhibits containing synthetic cannabinoids are likely to be marketed in ways that are similar to other psychotropic plants, such as marijuana or salvia divinorum. However, synthetic cannabinoids are manufactured and then applied to plant material; they do not occur naturally within the matrices in which they are commonly found. Thus variables exist that could affect the analysis of synthetic cannabinoids that are

extracted from a matrix, which may not be considered when analyzing other plant-based drugs of abuse in which the drugs are naturally-occurring. For example, little appears to be understood about the effects of burning on synthetic cannabinoid detection. For forensic purposes, such a variable is useful as some exhibits that are submitted for analysis to controlled substances laboratories may already have been used, or smoked. Plant material samples suspected to be marijuana are frequently submitted to laboratories for analysis in the form of cigarettes that have already been smoked. When a burned marijuana cigarette, known colloquially as a "roach", is analyzed for the presence of cannabinoids, results can sometimes differ from those obtained from unburned material. These differences are evident in presumptive tests, such as the Duquenois-Levine test, and also in chromatographic tests, including thin layer chromatography (TLC). While the natural cannabinoids in marijuana can be detected after having been burned, whether or not the same effect would be observed in synthetic cannabinoid products is not known. Additionally, components within the matrix of the herbal products, such as botanical interferants, or other illicit substances present at different concentrations, could potentially affect the detection and analysis of the cannabinoids. It is the objective of this thesis, therefore, to

determine any differences, using a semi-quantitative technique, between peak areas of synthetic cannabinoids in solution and within a matrix.

II. Materials and Methods

1. Synthetic Cannabinoids

This study primarily focused on one target analyte, 1-(5-fluoropentyl)-3-(1-naphthoyl) indole (AM-2201). Additional cannabinoids were also used, including (2-methyl-1-propyl-1H-indol-3-yl)-1-naphthalenyl methanone (JWH-015), 3-(1,1-dimethylheptyl)-6aS,7,10,10aS-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol (HU-211), and (4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone (RCS-4). All of these compounds were purchased from Cayman Chemical. The structures of these cannabinoids are referenced in Figure 5.

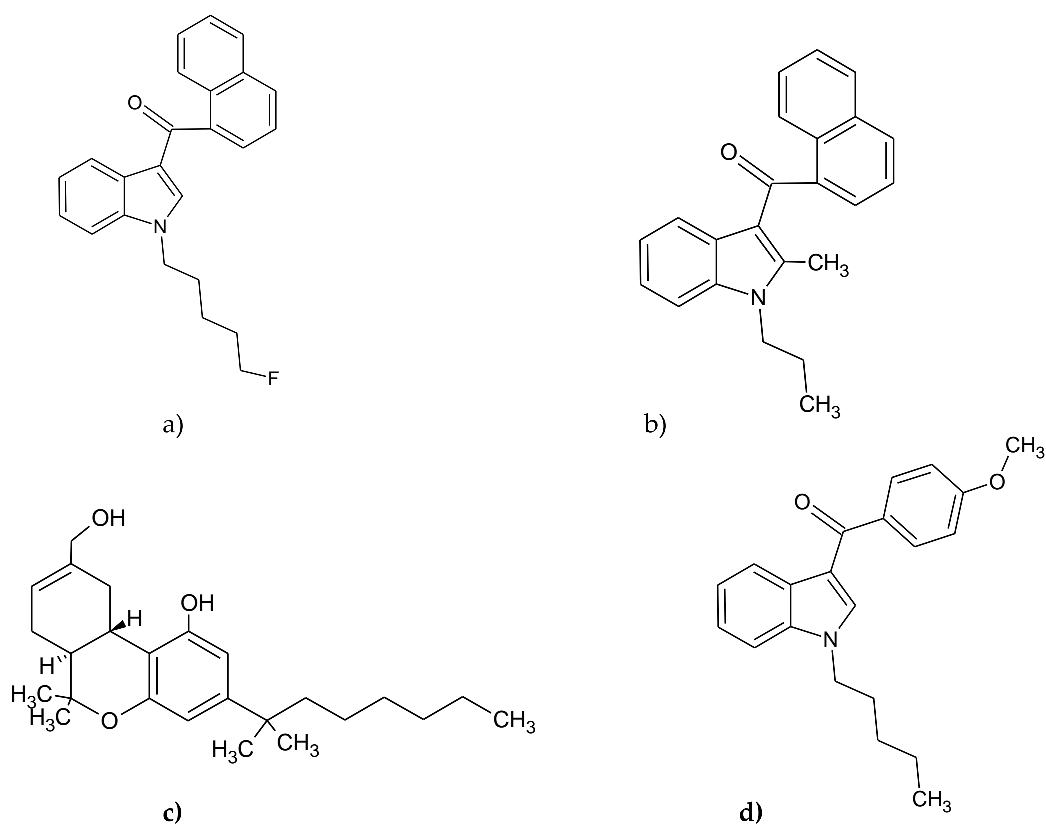


Figure 5. Structures of synthetic cannabinoids investigated: a) AM-2201, b) JWH-015, c) HU-211, and RCS-4 respectively.

2. Substrates

Because synthetic cannabinoids are used to spike various plant materials, the substrates used for these experiments were chosen liberally. As the results from the experiments are exclusively focused on the analysis of synthetic cannabinoids, neither the type nor amount of substrate used was optimized. The substrates used were instead chosen based on the ingredients or components that may be found in some herbal products on the market. One of the more popular synthetic cannabinoid products, "Spice," has been reported to contain *Scutellaria*

nana, a member of the Lamiaceae plant family. Two substrates used for analysis were therefore common members of the Lamiaceae family: mint and rosemary.[40] Additionally, rolling paper and tobacco were selected as substrates that were not botanical members of the Lamiaceae plant family. As a result of using different substrates, the number of components that potentially interfere with the detection of target cannabinoids also varies; a more detailed evaluation is needed regarding the potential effects of substrate properties on cannabinoid detection.

Tobacco was purchased from a local merchant located in Boston, Massachusetts, and was the "Smoker's Delight," brand. The paper that was used was Bambu brand rolling paper and purchased from the same local merchant located in Boston, Massachusetts as the tobacco samples. Both mint and rosemary were packaged by McCormick (Gourmet Collection), and were purchased from Shaw's Supermarket.

3. GC/MS Conditions

The primary method of analysis was gas chromatography/mass spectrometry (GC/MS) analysis. The instrumentation consisted of an Agilent Technologies 7890A gas chromatography (GC) system with a DB-5MS capillary column (30m x 0.25mm x 0.25µm) in tandem with an Agilent Technologies 5975C MS. All spectra were analyzed using Agilent™ ChemStation software.

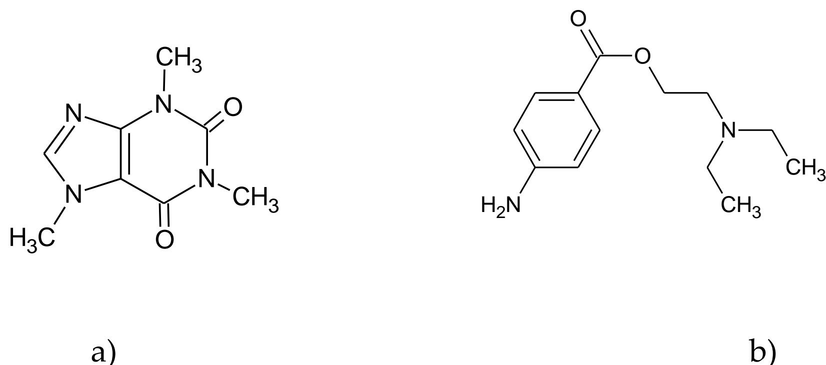
III. Experimental Design

1. Optimization Studies

1.1 Optimizing a Burning Procedure for Synthetic Cannabinoid Analysis

At the time of these experiments, published procedures for the burning of synthetic cannabinoids, or of any of the products containing synthetic cannabinoids, were not discovered. Therefore, the burning procedures used for the interests of these experiments were developed as part of the research conducted. A proof of concept study was conducted in order to establish a methodology that provides an adequate burning technique.

For the proof of concept study, two readily available and inexpensive compounds were used: caffeine and procaine. These compounds are not structurally similar to the cannabinoids that were examined, so the results obtained from these experiments were only used as confirmation that the burning procedure was one that would allow detection of the caffeine and procaine, with little risk for loss. By extension, therefore, it was postulated that the burning procedure established in these studies could be used for the burning of the synthetic cannabinoid samples. The chemical structures of these compounds are shown in Figure 6.



a) b)

Figure 6. Structures of a) caffeine and b) procaine.

Instead of applying direct flame to the compounds of interest, they were exposed to heat after they were transferred to a crucible. Because heat is a crucial aspect of fire, this is one factor that can potentially affect the detection of AM-2201. Approximately 2mg of caffeine and procaine were measured in Eppendorf tubes and dissolved in 1mL of methanol. These solutions were transferred to four, clean, dry, porcelain crucibles, and then evaporated to dryness at room temperature. A Bunsen burner flame was applied to two crucibles for 2 minutes, which were then allowed to cool. Methanol was then used to transfer all samples, whether unburned or burned, to clean vials. All of the samples were analyzed using a designed GC/MS method known as the "BAS.m" method. The parameters of this method are outlined in Table 1.

Table 1. Parameters of BAS.m Method

Initial Temperature	50°C
Ramp rate	30°C/minute
Final hold temperature	280°C
Final hold time	4 minutes
Total experimental run time	12.3 minutes

1.2. GC/MS Method Optimization

While the BAS.m method was used for the proof of concept study, the method parameters were not ideal for the analysis of synthetic cannabinoids. Specifically, the final temperature of the method was lower than published temperatures for GC/MS methods used to analyze synthetic cannabinoids, and the retention time for synthetic cannabinoids was found to generally be higher than the slow ramp rate warranted. Therefore, optimization was a prudent and necessary aspect of the preliminary studies.

The method that was designed for the optimized detection of AM-2201, AM2201SIMHM, was adapted from Agilent Technologies. The parameters of this modified method are summarized in Table 2.

Table 2. Parameters of AM2201SIMHM Method

Initial Temperature	100°C
Ramp rate	50°C/minute
Final hold temperature	300°C
Final hold time	8 minutes
Total experimental run time	13 minutes

1.2.1. Selective Ion Monitoring (SIM) Methodology

The final GC/MS method used, AM2201SIMHM, was also programmed to collect selective ion monitoring (SIM) data. Most GC/MS analyses are performed using scan methods, where the instrument is programmed to look for every ion within a specific mass range. The resulting mass spectra are commonly used to compile databases for identification of unknown compounds. Spectra from the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) mass spectral library for the compounds analyzed are shown in Figure 7.

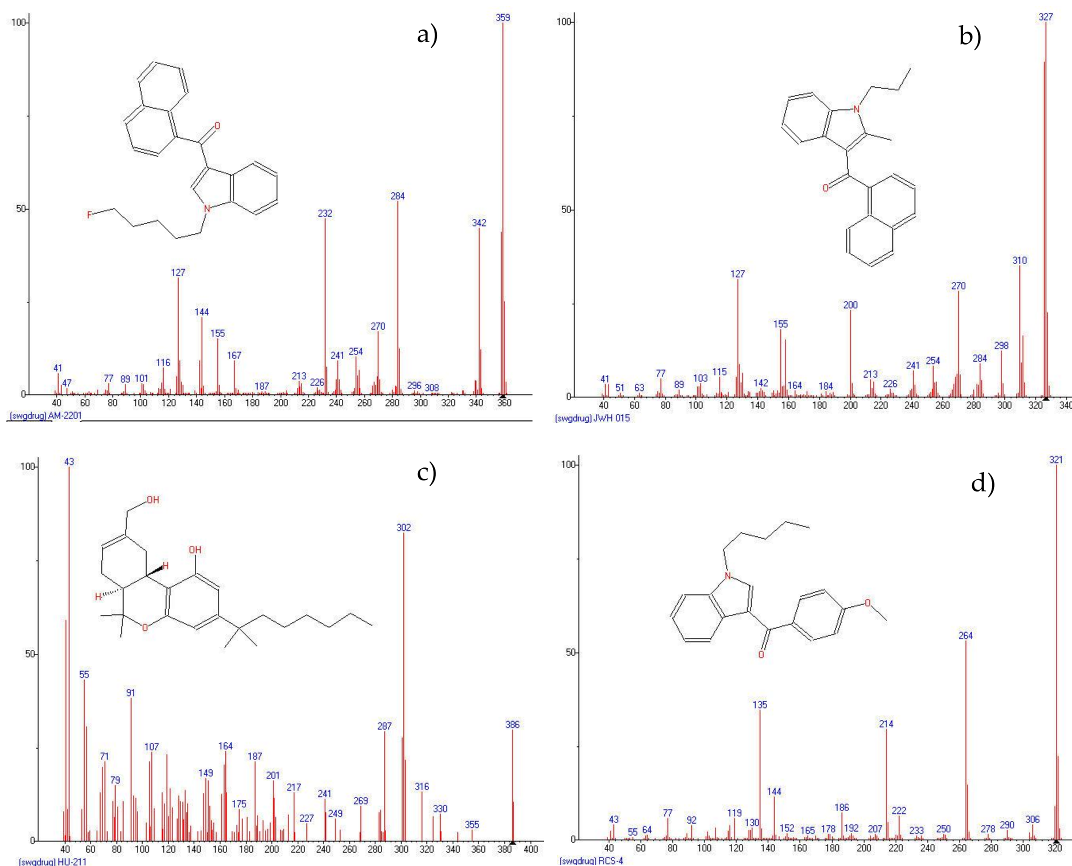


Figure 7. Mass spectra of AM-2201 (a), JWH-015 (b), HU-211 (c), and RCS-4 (d) from the SWGDRUG database[43]

Methods can also be programmed, however, to collect SIM data, where fragment ions of only one or two m/z ratios are selected. Although SIM methods can be less accurate than scan methods, due to the identification of unknown compounds based on one ion rather than several, they are advantageous due to the increased sensitivity. The detection limit is generally lower using a SIM method because instead of detecting a range of fragment ions, an exclusive fragment ion is targeted and all detection capability is focused on this selected fragment, despite the concentration of the analyte remaining constant between methods.[42] The use of SIM methods in forensic analysis can be beneficial due to the increased sensitivity afforded in comparison to scan methods. SIM methods are ideal when analyzing samples that contain sources of possible contamination, because known fragment ions are selected, increasing the possibility of detecting the target analyte. Utilizing a SIM method also allows detection of a target analyte if it is at a relatively lower concentration than other unknowns within the sample. SIM is not without disadvantages, however. If fewer ions are targeted, the identity of the compound becomes more dependent on the retention time observed from the gas chromatogram. Hence while analytes can be more easily detected within a mixture using SIM methods, other constituents within a mixture that possess a similar fragment ion as the one attributed to the target

analyte could therefore contribute to the target analyte peak area if the gas chromatogram demonstrates peaks that are not entirely resolved.[27]

The AM2201SIMHM method was programmed to select the molecular ion of AM-2201, 359 amu. Additionally, the AM2201SIMHM method was used as a template to design SIM methods for the other synthetic cannabinoids that were studied. The molecular weights of JWH-015, HU-211, and RCS-4 (327g/mol, 386g/mol, and 321g/mol respecitively) were all used as the selected ion for the individually designed SIM methods. The molecular weights were selected as the target ions as they are unique to each of the specific cannabinoids.

2. AM-2201 Standard Analysis

2.1 Unburned, "Neat" AM-2201 Standard Analysis

Unburned, or "neat," samples of AM-2201 were prepared according to the procedure outlined in the proof of concept study. Although not all the samples were burned, all samples were transferred to crucibles in order to maintain consistency in the preparation procedure. This also minimized the potential decrease in detection that would simply be caused by the transferring process.

A 2.8mg/mL solution of AM-2201 was prepared in an Eppendorf tube using methanol. This solution was transferred into a clean, dry crucible and evaporated to dryness. After evaporation, the original stock concentration was

re-constituted using methanol. This method was repeated a total of three times. Various concentrations were made from this stock solution, which included 25, 50, 75, 100, 150, 200, and 250ug/mL. These concentrations were all analyzed using the specified AM2201SIMHM GC/MS method.

2.2. Burned AM-2201 Standard Analysis

Due to limited studies related to the effect of burning on synthetic cannabinoid extraction and subsequent analysis, little is further known regarding a distinct difference between the chemical properties of unburned and burned synthetic cannabinoids. Specifically, no published research was found regarding whether or not the act of burning AM-2201 will result in pyrolysis of the compound. In order to determine whether AM-2201 pyrolyzes in response to burning, standards of AM-2201, without the presence of interfering plant substrates, were burned. This examination therefore aimed to investigate the effect of burning on the detection of AM-2201, and subsequently whether or not the possible differences between results of AM-2201 analysis could be exclusively due to the chemical transformation of the compound rather than due to other variables introduced due to a matrix.

The procedure for these experiments was established in the proof of concept study, though the results from this study were not directly used to form

conclusions regarding the relative differences in detection due to burning. A recorded mass of AM-2201 standard was measured in an Eppendorf tube, as with the unburned samples, and dissolved in 1mL of methanol. An aliquot volume containing 250ug was then transferred to a clean, dry crucible, and evaporated to dryness. This crucible was then heated using a Bunsen burner for two minutes and then cooled. After the crucible was cool enough to handle, the burned AM-2201 was then recovered using methanol, which was washed into vials. The methanol washes were then evaporated to dryness. A volume of 1mL was then added to each residue, and the resulting solution was used to prepare a series of eight concentrations. This method was performed three times.

2.2.1 Burning of Smaller AM-2201 Concentrations

Initially, the concentrations of AM-2201 that were analyzed were the same as those that were prepared using the unburned samples, consisting of 25, 50, 75, 100, 150, 200, and 250ug/mL. These concentrations were chosen in order to determine any trends or differences between the peak areas of equivalent concentrations of unburned AM-2201 and burned AM-2201. However, analysis of the burned AM-2201 samples showed no reproducibly detectable peak areas, as AM-2201 was not always detectable, and if a peak was present, it could not be integrated properly.

The final solutions of burned AM-2201 were prepared as described above. The concentrations of burned samples of AM-2201 that were analyzed were 500, 750, 1000, 1250, 1500, 2000, 3000, and 5000ug/mL. As with the unburned AM-2201 samples, these solutions were prepared, and analyzed, in triplicate using the "AM2201SIMHM" GC/MS method.

3. Analysis of AM-2201-Spiked Substrates

The substrates that were utilized as matrices, and thus hypothetical models of similar herbal products that contain synthetic cannabinoids included tobacco, paper, mint, and rosemary. Before AM-2201 was added to the individual substrates, extracts of both unburned and burned substrates were analyzed separately to compile a collection of GC/MS spectra to which the spectra obtained from analyzing the spiked plant material could be compared.

For the analysis of the substrate extracts, approximately 300mg of each substrate was measured and placed in one of two clean, dry crucibles. One sample was heated using a Bunsen burner for 2 minutes, or until the sample ignited. If ignition occurred, the sample was allowed to burn until the flame extinguished. After the crucible cooled, methanol was then added to both the burned and unburned substrates to immerse the sample, and was allowed to extract for 5 minutes. This extract was then transferred to vials, and left to

evaporate to dryness. A volume of 0.5mL of methanol was then added to the dried residues, which were subsequently analyzed.

For the analysis of the spiked samples, approximately 300mg of each substrate was placed in separate crucibles as described above, and a solution of 5000ug/mL AM2201 was added to each crucible containing the substrate. These procedures were also performed in triplicate, and all extracts were analyzed using the "AM2201SIMHM" method.

4. Analysis of AM-2201 Mixtures with Other Synthetic Cannabinoids

A key variable to consider for forensic analysis of synthetic cannabinoid products, in addition to possible interfering effects of components within a matrix, is the relative detection of one synthetic cannabinoid in the presence of others. Many herbal products are sold, and could potentially be submitted to laboratories for analysis, consisting of several cannabinoids. Other synthetic cannabinoids, therefore, were combined together in solution in order to determine whether or not the presence of other cannabinoids influence the detection of a targeted cannabinoid.

Stock solutions of AM-2201, JWH-015, HU-211, and RCS-4 were prepared in methanol. Specific volumes of each of these cannabinoids were combined in order to give 1:1:1:1 dilutions of each cannabinoid (each measuring 250ug/mL) in

a total solution of 0.2mL of methanol. This solution was then analyzed using the "AM2201SIMHM" GC/MS method, and the areas of each peak observed were recorded. This procedure was further expanded to include 1:2:2:2 (125ug/mL AM-2201, 250ug/mL JWH-015, HU-211, and RCS-4 respectively) solutions, and 2:1:1:1 (250ug/mL AM-2201, 125ug/mL JWH-015, HU-211, and RCS-4 respectively) solutions. These samples were also prepared in triplicate.

5. Analysis of Substrates Spiked with Various Synthetic Cannabinoids

Finally, the synthetic cannabinoid mixtures containing all of the investigated cannabinoids were used to spike substrates in order to evaluate the effect of both the presence of substrates and of other synthetic cannabinoids on the detection of each cannabinoid present in the matrix. The prepared 1:1:1:1 mixtures of synthetic cannabinoids were used to spike the same substrates as previously described: tobacco, paper, mint, and rosemary.

IV. Results and Discussion

1. Unburned "Neat" AM-2201 Analysis

The relationship between the peak areas of unburned AM-2201 standards with concentration is depicted in Figure 8.

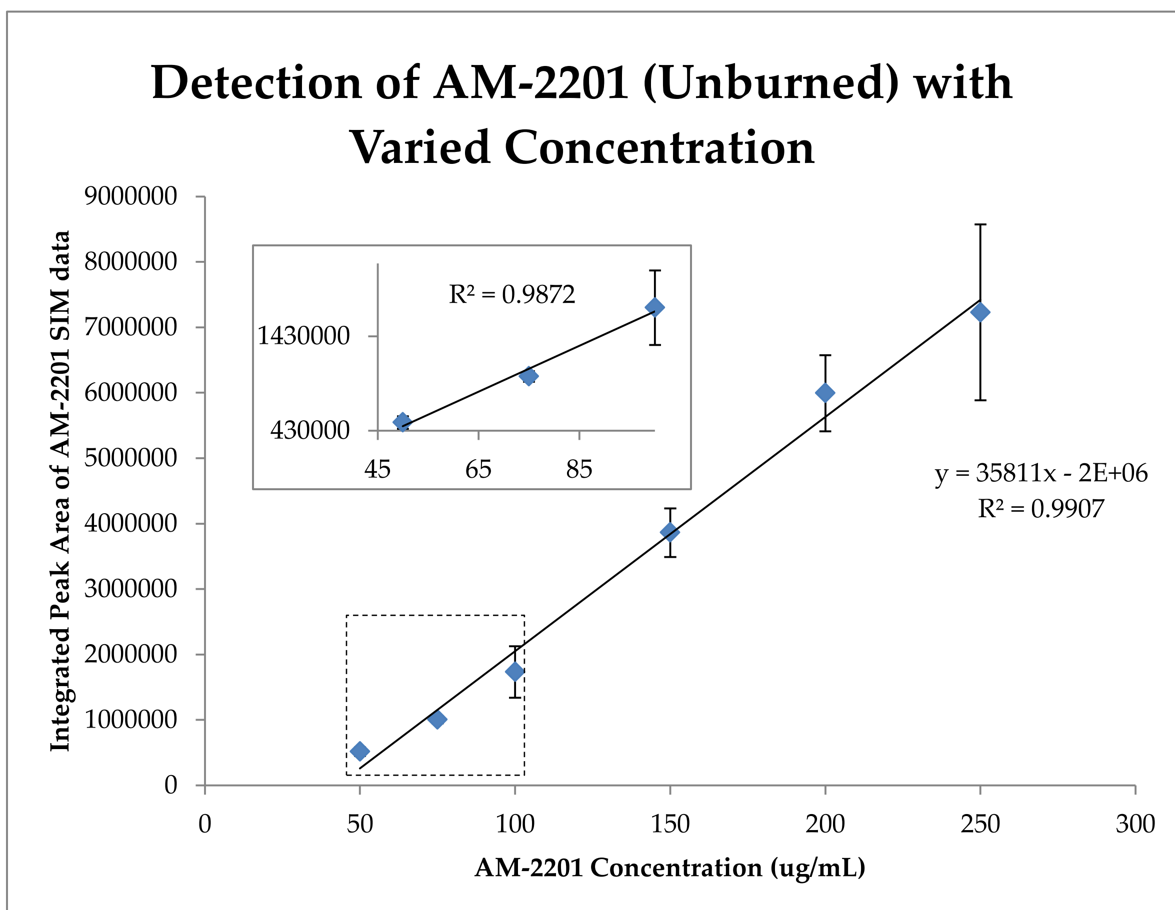


Figure 8. Relationship between concentration of unburned AM-2201 and the peak area represented by a 6-point calibration curve. The inset shows the lowest concentrations plotted separately, with an individual R^2 value.

From these data, the peak area of AM-2201 appears to have a direct relationship with concentration if the 25ug/mL and 75ug/mL data is eliminated as outliers. This suggests that the relationship may not be as linear in a more narrow range, which is substantiated by the inset in Figure 8, where the R^2 value decreases. However, there is not a significant deviation in linearity at the three lowest

points; therefore, the linearity is shown to be maintained at the lower concentrations, and within this narrower range. It is likely, however, that traces of AM-2201 remained on the crucibles from which the AM-2201 was transferred, which would become a more significant source of error at lower concentrations than higher ones. This potentially caused more error to be accumulated due to the number of steps used in the sample preparations. The sample was transferred several times; therefore, it is possible that some sample remained after each step of the procedure, thus providing a possible source of significant experimental error.

An additional source of error includes the fact that AM-2201 was not derivatized prior to GC/MS analysis. While sources of experimental error contributed to the high standard deviations within any one concentration, the absence of derivatization of a polar compound can decrease the detection capabilities of GC/MS.

2. Burned AM-2201 Analysis

The relationship between peak area and concentration of burned AM-2201 standards is depicted in Figure 9.

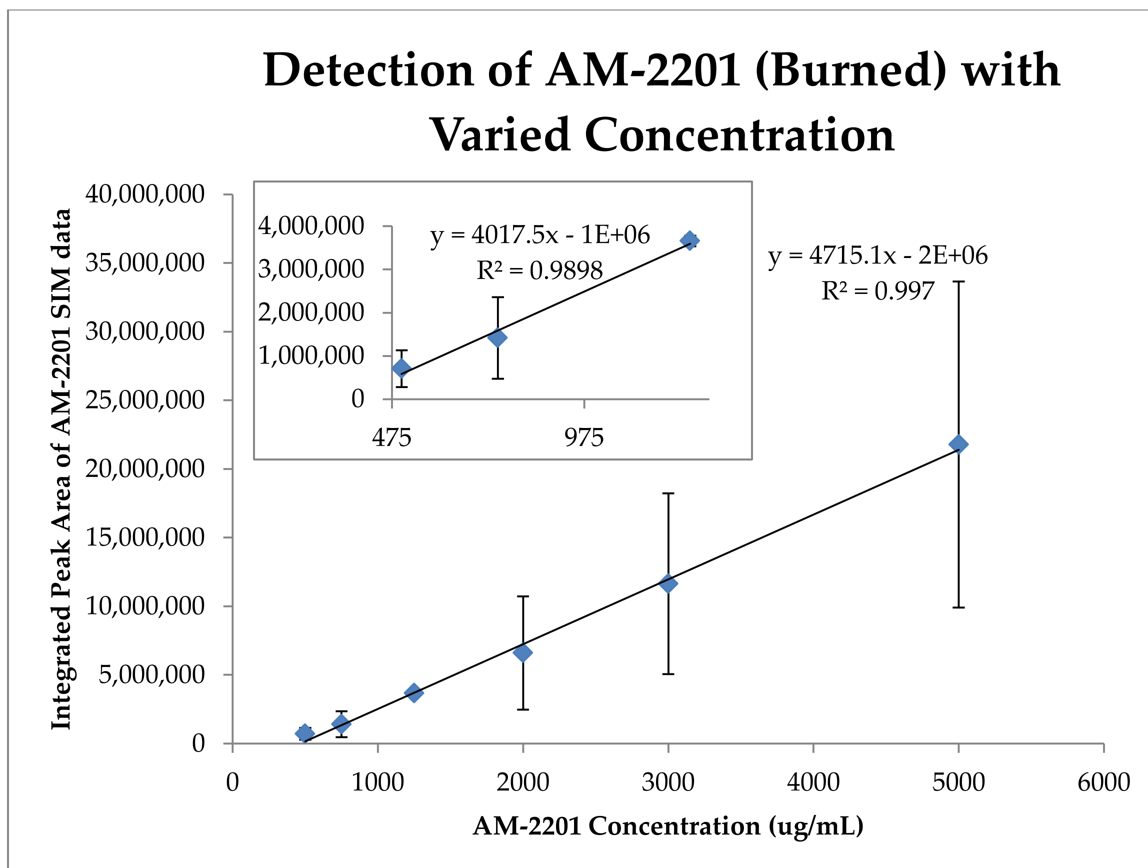


Figure 9. Relationship between concentration of burned AM-2201 and the peak area represented by a 6-point calibration curve. The inset shows the lowest concentrations plotted separately, with an individual R^2 value.

As observed with unburned AM-2201, the peak area for AM-2201 observed in the SIM data increases with concentration, suggesting that the dependent relationship is maintained despite the burning of the sample. Interestingly, the percent standard deviation of the triplicate measurements for the burned data is more pronounced in the highest concentration, which was also observed in the unburned data. Sources of experimental error, such as analyte

loss from transferring from the crucibles may contribute to the error here, but a source of variability that could contribute to the high standard deviations for burned compounds specifically relates to the combustion of the AM-2201. As previously established in the preliminary studies, higher concentrations were needed for the burned experiments as concentrations that were previously detectable as unburned compounds were not after burning. This suggests that the AM-2201 degrades, sublimes, or is otherwise consumed by the process of combustion induced by extreme heat from the Bunsen burner. If this rationale is accurate, based on the results conveyed within Figure 9, the degradation of AM-2201 is inconsistent between the triplicate measurements at any given concentration for the burned samples, which consequently leads to inconsistent peak areas. Further knowledge would need to be obtained, however, on the chemical and physical properties of this compound in order to form a definitive conclusion.

As previously mentioned, the standard deviations could be high because the samples were not derivatized before analysis. The mechanism and product of thermal degradation for AM-2201 is unknown; thus derivatization of a burned sample of AM-2201 could vary slightly from derivatization of an unburned sample. Further research and information regarding the degradation of AM-

2201 would need to be obtained to fully understand the benefits of derivatizing burned synthetic cannabinoids.

3. Effect of Burning on AM-2201 Peak Area

The correlation between the peak area and the concentration of AM-2201 can be represented by the equations of the linear trendlines.

The equations of the line for the unburned (U) and burned (B) standards of AM-2201 are shown below.

$$y_{(U)} = 35{,}811x_{(U)} - 2E{+}06$$

$$y_{(B)} = 4715.1x_{(B)} - 2E{+}06$$

These equations can further be combined to produce the following expression:

$$y_{(U)} - y_{(B)} = 35{,}811x_{(U)} - 4715.1x_{(B)}$$

$$y_{(U)} - y_{(B)} = 35{,}811\,(x_{(U)} - 0.132x_{(B)})$$

Consequently, if the AM-2201 concentrations are equivalent, the correlation between the difference of peak areas and concentration of burned and unburned AM-2201 is:

$$y_{(U\text{-}B)} = 35{,}811\,(1 - 0.132(x_{(U,\,B)}))$$

$$y_{(U\text{-}B)} = 35{,}811\,(0.869(x_{(U,\,B)}))$$

$$y_{(U\text{-}B)} = 31{,}096(x_{(U,\,B)}) \pm 29{,}894$$

Therefore, the difference in the peak area for equivalent concentrations of AM-2201 (burned and unburned) can be approximated to be 31,096 times.

Similarly, if the equations are combined, a relationship between the concentration of burned AM-2201 ($x_{(B)}$) and unburned AM-2201 ($x_{(U)}$) is calculated to be:

$$35811x_{(U)} - 2E{+}06 = 4715.1x_{(B)} - 2E{+}06$$

OR

$$4715.1x_{(B)} = 35811x_{(U)}$$

This simplifies to:

$$x_{(B)} = 7.59x_{(U)} \pm 11.01$$

where the peak area terms for burned and unburned standards are considered equal. This equation mathematically demonstrates that in order to achieve comparable peak areas, a burned sample of AM-2201 is required to be analyzed in a solution that is at least 8 times more concentrated than the respective unburned solution. This relationship further suggests that while the limits of detection were not specifically identified, the limit of detection of burned AM-2201 would be expected to be significantly higher than that of unburned AM-

2201. A possible explanation is that the majority of AM-2201 in a given sample is degraded by the extreme heat applied to the sample.

4. AM-2201 Unburned Matrix Samples

The peak areas obtained from spiked substrates were divided by the peak areas obtained from analyzing pure solutions of AM-2201 in order to determine a percentage between the two values, therefore obtaining normalized values that are suitable for comparison between the substrates. The lower percentages represent the greatest difference between the peak area observed from analyzing standard AM-2201 to analyzing AM-2201 when present in a matrix. These results are summarized in Figure 10.

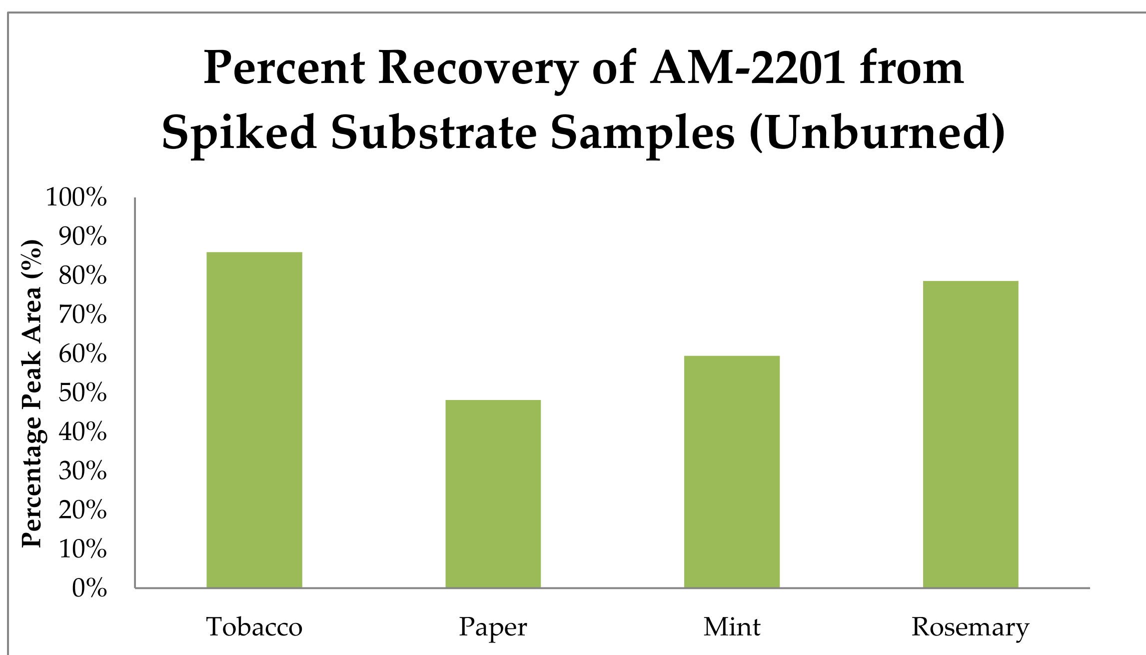


Figure 10. Proportion of AM-2201 observed from 250ug/mL present in a matrix compared to 250ug/mL neat solution. These experiments were performed in triplicate.

The presence of a substrate appears to cause a variation between the peak areas of AM-2201. This could be due to various factors, such as differing adsorption affinities between the substrate and the AM-2201. If a given substrate demonstrates a higher adsorption affinity for AM-2201 compared to another, the recoverability of the AM-2201 is then diminished. Upon comparing the peak area percentages of all the substrates, paper was found to be the substrate that yielded the lowest percent peak area. Paper, as a non-botanical substrate, would not have the same adsorption affinities, or other physical properties, as the other substrates that are unprocessed plant material. The decrease in percent peak area is therefore more likely due to differences in AM-2201 affinity for the botanical substrates. If this hypothesis were valid, there would be evidence of such differences found in the peak areas of AM-2201 obtained from spiked substrates and those obtained from pure unburned AM-2201.

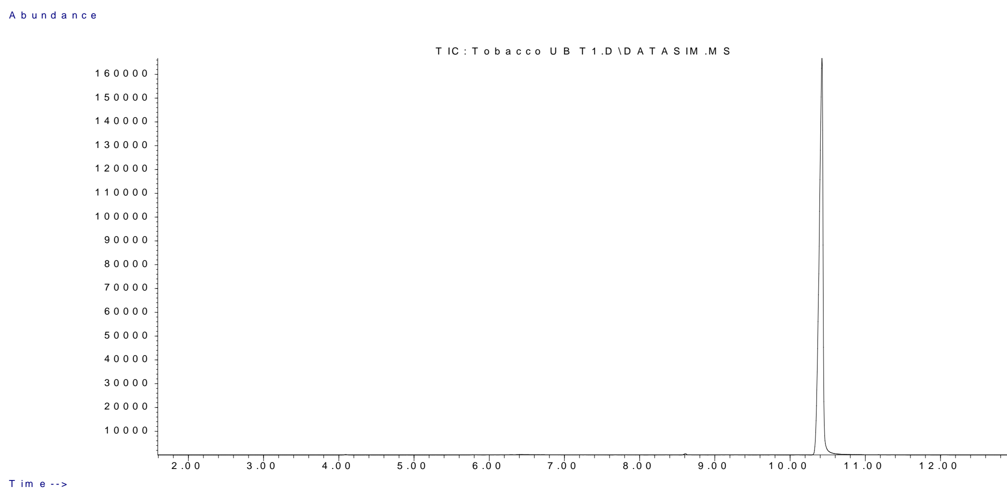
The peak areas obtained from the analysis of paper extracts were also found to demonstrate the largest standard deviation. These deviations are likely due to the lack of reproducibility that was caused by significant analysis considerations and variations that were encountered during the sample preparation. AM-2201 that was used to spike tobacco was found to have the

highest percentage of AM-2201 peak area, which suggests that most of the AM-2201 was successfully extracted from the substrate, possibly due to the tobacco demonstrating a lower affinity for AM-2201.

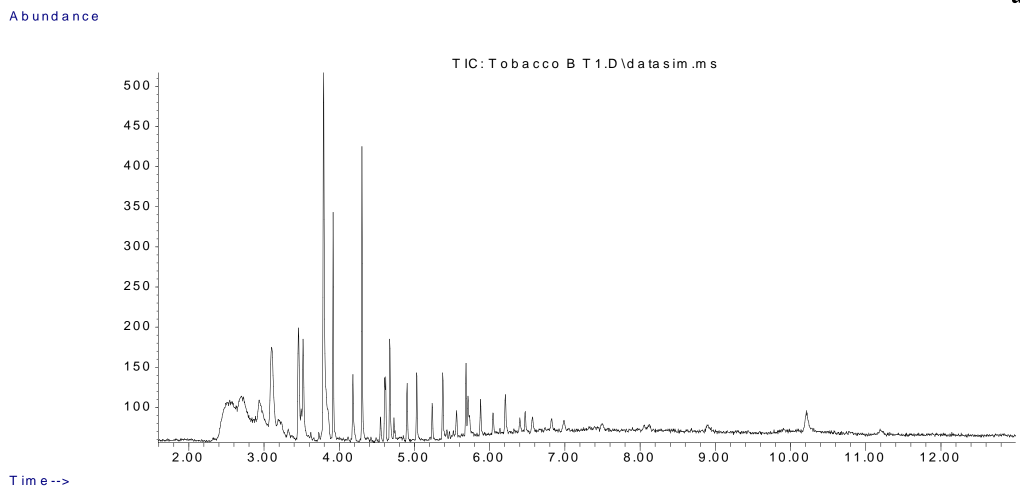
5. AM-2201 Burned Spiked Matrix Samples

An AM-2201 concentration of 250ug/mL was previously determined to not be detectable after burning; therefore 500ug/mL solutions were used to spike substrates that were then burned. Despite an increase in concentration, however, AM-2201 could not be detected in extracts from the burned spiked samples.

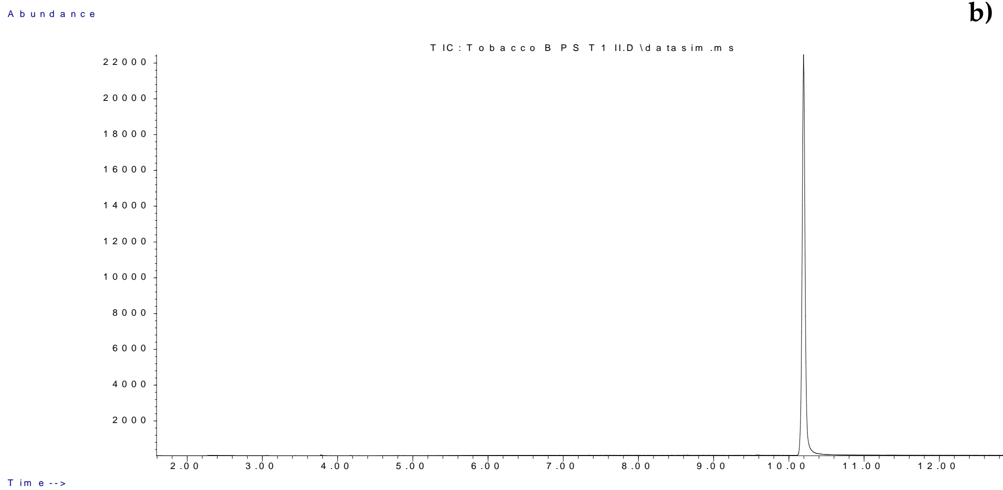
As observed from the analysis of unburned substrates that had been spiked with AM-2201 (Figure 9), varying degrees of affinity for AM-2201 appear to be based on the substrate. In order to investigate whether or not burned substrates also have different degrees of AM-2201 affinity, a supplemental series of experiments was designed and performed. All substrates were burned, and then subsequently spiked with a 500ug/mL solution of AM-2201, extracted, and then analyzed by GC/MS.



a)



b)



c)

Figure 11. AM-2201 SIM data for a) unburned, b) burned (after spiking), and c) burned (before spiking) tobacco.

The results from analyzing extracts that were spiked after burning were used to demonstrate that the loss of analyte observed is due to the burning and combustion process alone, and not due to a greater AM-2201 affinity for burned substrates.

AM-2201 was successfully extracted from all burned substrates, which were subsequently spiked, and analyzed. This infers, therefore, that the significant decrease in peak area for AM-2201 in a burned spiked substrate is more likely due to the burn procedure rather than variables related to the substrates. SIM data for all tobacco extracts of AM-2201 (from unburned tobacco, burned tobacco, and tobacco that was burned before spiking the substrate) are exhibited in Figure 11. Results from all matrices demonstrated similar results; most importantly, no results from extraction of other matrices demonstrated evidence that the presence of burned matrices significantly affected the analysis of AM-2201.

There appears to be a decrease in peak area in Figure 11, part c), which could be due to interactions between AM-2201 and the burned matrix, but the extreme decrease in peak area observed in Figure 11, part b) is most likely due to effects ensued from burning. A concentration that is known to be detectable when AM-2201 is burned (500ug/mL) would no longer be detectable if burned

with a substrate because the substrate provides a fuel for the burning, which causes the heat to increase. The increased heat then causes more AM-2201 to be degraded or sublimed, compared to what is observed from heating a pure compound. When pure AM-2201 was heated, smoke was observed, indicating combustion, but when the substrates were heated, ignition occurred. This observation further adds to the theory that faster, or more intense, combustion may cause AM-2201, and by extension other cannabinoids, to be more difficult to detect.

6. Cannabinoid Mixture Analysis

It is unlikely that an herbal product containing synthetic cannabinoids that is submitted for analysis would contain only one cannabinoid, as products that have been analyzed all contain several cannabinoids.[17] It is therefore useful to investigate the possible effect of the presence of other synthetic cannabinoids as another variable.

Synthetic cannabinoids are frequently found in herbal products at varying concentrations.[21] As previously discussed, the relationship between concentration and peak area is direct and linear over a moderate range of concentrations. Mixtures of AM-2201, JWH-015, HU-211, and RCS-4 were combined together to determine whether or not this linear relationship would be

maintained for any specific cannabinoid in the presence of other cannabinoids. AM-2201, JWH-015, HU-211, and RCS-4 were combined together to make a 1:1:1:1 mixture of each cannabinoid at a concentration of 250ug/mL in 0.2mL of methanol, and was subsequently analyzed. The concentration of each cannabinoid was then doubled to 2:1:1:1 (in relation to JWH-015), and each solution was analyzed using individual SIM methods for every cannabinoid. The results from these tests are shown in Figure 12. Peak areas of HU-211 are featured again in Figure 13 in order to observe the results clearly.

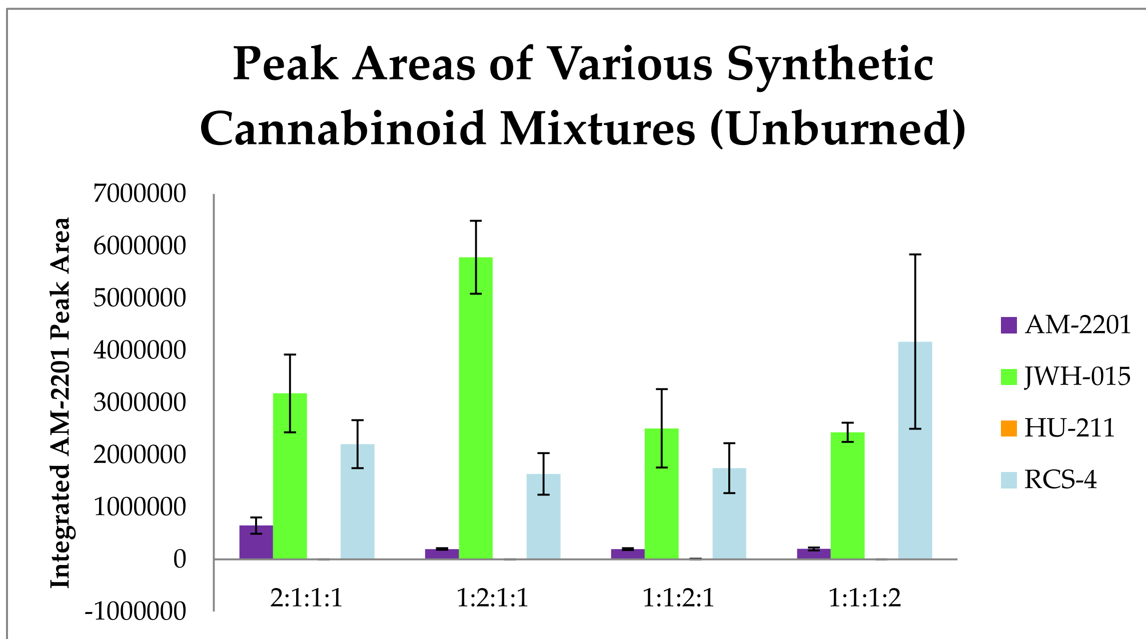


Figure 12. Peak areas of various synthetic cannabinoids in solution, varying from 250ug/mL to 500ug/mL.

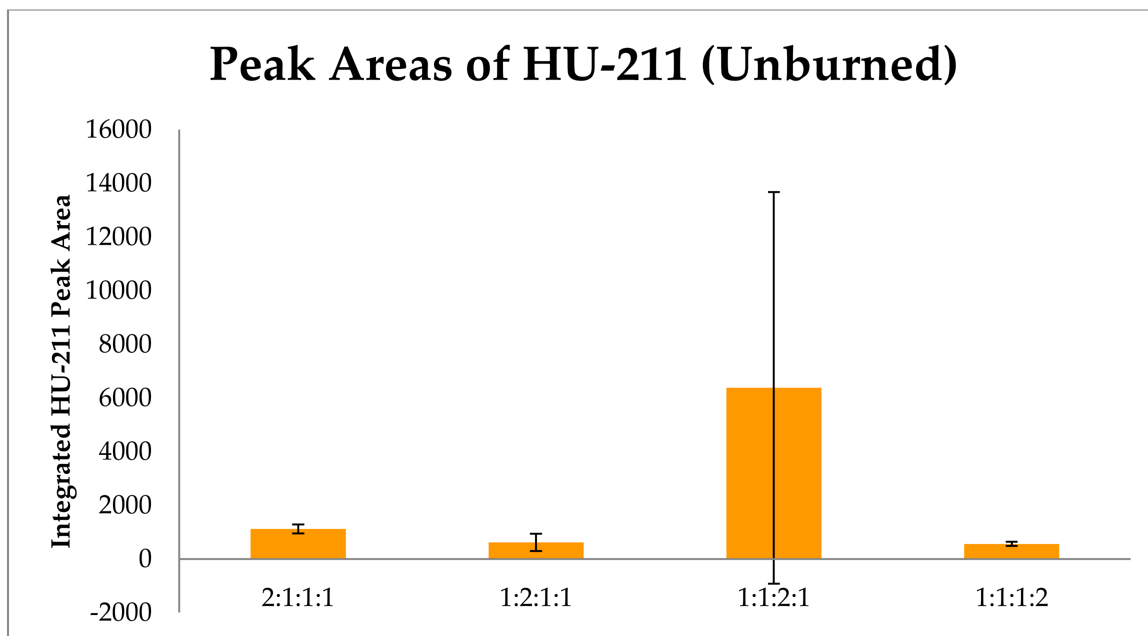


Figure 13. Peak areas of HU-211 in solution with other synthetic cannabinoids (not shown).

Two important aspects can be derived from these data. First, there is a wide range of peak area variation among the different synthetic cannabinoids, despite being at equivalent concentrations. For example, HU-211 is the cannabinoid that was the most difficult to detect, as the associated peak area was vastly smaller than those of the other cannabinoids. Second, the doubled concentration increased the peak area of the respective cannabinoid in all cases, but the degree to which doubling the concentration affected the peak areas of each cannabinoid relative to one another did not remain consistent. For example, AM-2201 exhibited a peak area that was smaller than either JWH-015 or RCS-4, whether it was twice the concentration or not. The peak area of RCS-4, however, surpassed that of JWH-015 when the concentration was doubled, having been lower in all

the other solutions. One important consideration is that although these concentrations are equivalent, the concentrations are based on mass; therefore the absolute number of molecules per unit of volume is not equivalent between cannabinoids. The differences in the number of molecules between the cannabinoids in a GC injection of a given volume could then have potentially contributed to the differences in peak area response. However, as the cannabinoids only differ slightly in molar mass, it is more likely that the differences in response are due to differences in physical properties, which include differences in volatility, thermal stability, and ionization efficiency. All of these physical properties would be expected to be analyte-dependent, and therefore, the differences observed are best explained by a thorough understanding of the chemical and physical properties of these compounds.

As increasing the concentration results in varying effects among different cannabinoids, varying the substrate onto which a cannabinoid mixture is spiked could also demonstrate differing peak areas. A 1:1:1:1 solution of the cannabinoids was used to spike the following substrates: tobacco, paper, mint, and rosemary, to observe the different peak areas obtained from each cannabinoid when applied to a different matrix. The sum of all peak areas from all cannabinoids from each substrate was calculated, and the individual peak

areas from each cannabinoid were divided by this total to give a percentage of the peak area for each cannabinoid. These percentages were then compared to those from a 1:1:1:1 neat solution of a cannabinoid mixture. These results are shown in Figure 14.

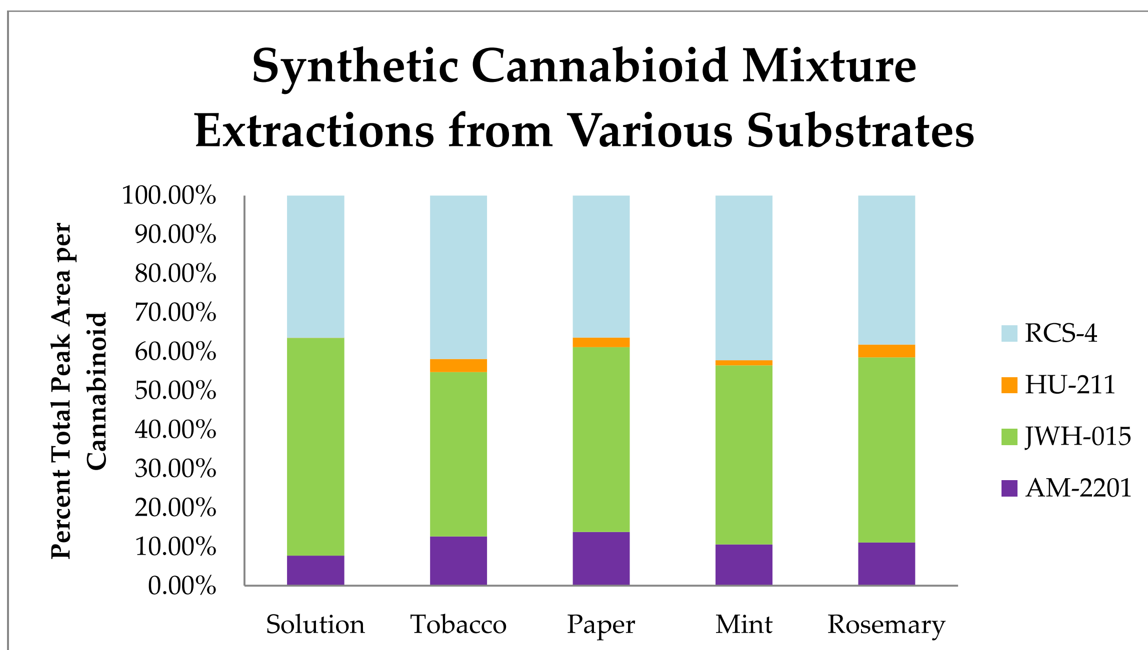


Figure 14. Proportions of peak area from each cannabinoid in a 1:1:1:1 solution, and in various spiked substrates. These experiments were performed in triplicate.

In general, the peak area percentages of each synthetic cannabinoid remain consistent with each substrate. Interestingly, however, HU-211 appears to be more detectable when extracted from a spiked substrate, as shown from the increased peak area. Differences in HU-211 affinity could have contributed to the lower peak areas of HU-211 observed from the analysis of cannabinoid

mixtures compared to peak areas observed from the analysis of the substrate extracts. However, the drastic differences observed between the peak areas of HU-211 and the other cannabinoids are attributed to a lower instrument detection sensitivity.

Another relevant trend is that associated with the standard deviation of the peak areas observed from the error. Although the standard deviations of the peak areas remain relatively small for cannabinoid solutions and tobacco extracts, those for the other substrates appear to be significantly larger. This could be due to the different affinities demonstrated by the individual substrates.

IV. Conclusions

There were three main variables that were explored in these studies that were thought to possibly affect the detection and analysis of synthetic cannabinoids: the effects of burning, the presence of a matrix, and the presence of other synthetic cannabinoids. All variables represent possible sources of difficulty when analyzing herbal products that contain synthetic cannabinoids. Upon consideration of all of the results obtained from these experiments, all three variables appear to affect the facility of cannabinoid analysis. Burning cannabinoids resulted in a decrease in peak area, which was likely due to degradation of the analyte from the applied heat. The results from analyzing

synthetic cannabinoids combined with substrates demonstrated that there was also an observable decrease in peak area when synthetic cannabinoids were introduced to substrates. Additionally, there was an observed trend of decreased peak areas between individual substrates. These decreased peak areas are most likely due to varying affinities between different cannabinoids and respective substrates. The presence of other synthetic cannabinoids appears to also affect the analysis of target analytes, particularly when analysis is done using SIM methods. Differences in physical properties, including volatility, thermal stability, and ionization efficiency, between individual synthetic cannabinoids possibly contribute to varying peak areas of each cannabinoid analyzed. It is recommended, therefore, that further research into all of these aspects should be continued.

V. Discussion and Future Directions

The purpose of these studies was to explore the potential difficulties of synthetic cannabinoid extraction and analysis by GC/MS that arise from cannabinoids being present in a matrix consistent with materials that would be smoked. Although the results suggested that the burning of synthetic cannabinoids and the presence of a substrate affects the detection of synthetic

cannabinoids, there are many areas that could be explored to further understand these effects.

This thesis focused on four cannabinoids and four types of substrates. Clearly, conclusions regarding any observed effects would have more significance if a wider array of synthetic cannabinoids were studied, and if more substrates were used. Furthermore, if the more popular synthetic cannabinoids, such as the cannabinoids that were emergency-scheduled, or if the commercial products containing the scheduled synthetic cannabinoids could be studied, the data could be immensely useful.

The effect of burning on the detection of synthetic cannabinoids was studied, but there are many opportunities to continue the work performed. AM-2201 was detectable when extracted from a substrate following burning, so it is therefore likely that other synthetic cannabinoids would be detectable following the same procedure. However, a more detailed investigation of this area could be pursued, where higher concentrations of various synthetic cannabinoids (separate or in a mixture) are burned in attempt to determine a limit of detection (LOD) for specific synthetic cannabinoids after burning. Furthermore, the decrease in detection after burning, and how it changes with different substrates that are spiked could possibly be explored.

The substrates were chosen based on botanical similarity to some ingredients found in commercial products that contain synthetic cannabinoids. However, characteristics of materials found in the illicit products containing scheduled cannabinoids could be significantly different than those of the substrates that were analyzed. It would be beneficial, therefore, to study herbal incense products that are sold on the market. Similarly, there could be benefit to studying more complex mixtures of synthetic cannabinoids, where both the type and amount of substrate is varied in addition to the type and number of other synthetic cannabinoids present in the mixture.

Another aspect of analysis that could be further investigated is the possible benefit of derivatizing AM-2201 or other synthetic cannabinoids before conducting analysis using GC/MS. Synthetic cannabinoids contain polar functional groups, which decrease the efficacy of GC/MS as an analytical method. Agilent Technologies published several collections of spectra and methods that featured synthetic cannabinoids in solution, which were derivatized prior to analysis. Further studies should consider the benefits of derivatization, as the high standard deviations encountered in the data may have been avoided or decreased if the cannabinoids were derivatized before analysis. In addition to polar functional groups, the possibility also exists that synthetic

cannabinoids lack sufficient volatility to be analyzed by GC/MS. During initial stages of this study, the relationship observed in increasing peak areas of unburned and burned AM-2201 due to concentration appeared polynomial in nature when smaller concentrations ranges (initially from 10ug/mL of AM-2201 to 100ug/mL) were used. This trend could be due to the presence of active sites in the inlet of the GC/MS with high affinities for AM-2201, which would cause analytes to become trapped in the liner instead of successfully adsorbing to the column, which is necessary for analysis. However, a more likely explanation for the trend is that AM-2201 and other synthetic cannabinoids lack sufficient volatility for GC/MS analysis. If the cannabinoids are not adequately volatized, some of the analyte may not be detectable, and at lower concentrations, the percentage of analyte lost would be higher. Therefore the option of derivatization for the purposes of addressing polar functional groups and increasing volatility of the compounds, or other preparation procedures that could potentially improve the linearity of this trend, could be another area for further study. More specifically, derivatization would be highly beneficial for quantitation of synthetic cannabinoids in a given sample, or for other experiments where reproducibility is important.

A problematic area of these studies was the standard deviation of the peak areas. The standard deviation was high between triplicates (or showed significant deviation), which subsequently resulted in the high errors. Studies focused on optimizing experimental parameters to minimize these sources of high variability (which could possibly include derivatization procedures) could also greatly contribute to further development in this area.

As previously mentioned, the burn procedure was developed and utilized based on rational design and experimentation. Optimization studies of burning procedures would undoubtedly contribute to developing burning methods with fewer sources for error in cannabinoid analysis. Further investigation into variables that affect the manner of burning could also expand the research conducted to include how the detection of synthetic cannabinoids is affected by specific manners of burning. . Such variables that could be studied include, but are not limited to, temperature of the flame used, duration of time that the sample is exposed to heat or flame, and direct exposure of the sample to a flame, rather than applying heat.

The instrumental methodology used to analyze synthetic cannabinoids could also be a source of future developments. GC/MS was the only analytical method used for these studies, but other instrumentation could possibly be

useful for these analyses, such as FT-IR, or LC/MS, for example. LC/MS could be a preferred instrumental technique as previous research suggests that it has been successfully used to analyze components in commercially available herbal products containing synthetic cannabinoids.[21]

Submitted illicit substance exhibits commonly include burned products; therefore, the analysis of burned samples that contain synthetic cannabinoids is a relevant area of forensic research. Variables such as components present in burned plant material instead of unburned material, and the heat from the combustion of the plant material, can potentially have a significant effect on the detection of illicit substances within the samples. Unlike other illicit substances analyzed from plant material, however, synthetic cannabinoids are not naturally present in the product in which they are found. There are potentially many other factors, currently unknown, that could interfere with the analysis of cannabinoid-containing exhibits, all of which could be further pursued and studied in order to obtain a more detailed understanding of the effects on analysis of synthetic cannabinoids. Furthermore, as these products increase in popularity, and as more cannabinoids become commercially available, the high potential for botanical and chemical variability with the products will likely continue to fuel the research surrounding additional aspects of these compounds.

VI. References

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VII. Curriculum Vitae

HEATHER GRAY MOWATT

12-998 Riverside Drive, Port Coquitlam, BC, V3B 7Y4, Canada
Phone : (604) 552-4732 **Email:** hgm@bu.edu
Year of Birth : 1986

EDUCATION

Boston University, Boston, MA January 2013 (pending)
M.S. Biomedical Forensic Sciences

British Columbia Institute of Technology (BCIT), Burnaby, BC, Canada August 2010
Advanced Specialty Certificate (ASC) Forensic Chemistry

Queen's University, Kingston, ON, Canada June 2009
B.S (Honours). Chemistry

EXPERIENCE

Massachusetts State Police Laboratory Forensic Sciences Group July 2011 – February 2012
Intern

- Worked in the DNA unit of the Maynard-based laboratory
- Used LIMS system to optimize inventory (including reagents and chemicals) input for analysis
- Evaluated stutter percentages of different alleles found at various loci of case samples

Health Canada Drug Analysis Services February 2010 – August 2010
Co-op Student, Analyst

- Worked in the forensic drug analysis services laboratory (DAS) under Richard Laing
- Analyzed marijuana samples according to national standard operating procedures (SOPs) and issued Certificates of Analysis for hundreds of marijuana and resin samples to be used in court
- Composed Work Instruction documents and other related documents for QA/QC purposes
- Personally performed TLC on case samples and operated GC-MS (Agilent), and FID (Agilent) instruments for analysis
- Earned Certificate of Designation in Canada for Drug Analysis, permitting the analysis of controlled substances

Department of Chemistry, Queen's University May 2008 – August 2008
Summer Research Student supervised by Dr. Richard Oleschuk

- Researched and synthesized polymer monoliths for the application to protein analysis
- Personally prepared porous polymer monoliths (PPMs) for micro-analysis
- Performed ESI-MS to investigate enzyme digestion efficiencies and MALDI for further proteomic analysis

Department of Chemistry, University of British Columbia May 2007 – August 2007
Summer Research Student supervised by Dr. Colin Fyfe

- Synthesized zeolite crystals and performed subsequent analysis using solid state NMR and X-ray Diffraction
- Investigated the effect of temperature on extent of maturation and final structure of ZSM-5 zeolite
- Personally operated NMR and X-ray Diffractometer, in addition to related computer programs

PROJECTS

Graduate Research Project

Title: *Differences in Synthetic Cannabinoid Detection in Response to Common Interfering Variables*

Undergraduate Research Project

Title: *The Detection of Polycyclic Aromatic Hydrocarbons (PAHs) in Various Fish Species Using Fluorescence Spectroscopy*

AWARDS AND CERTIFICATIONS

Certificate of Designation, Health Canada May 2010 – present
Dean's Honour List, Queen's University April 2007
Dean's Honour List, Queen's University April 2006

PROFESSIONAL ASSOCIATIONS

American Association of Forensic Scientists (AAFS)

Registered Attendee February 2012
Student Member November 2010 – present

Northeastern Association of Forensic Scientists (NEAFS)

Registered Attendee November 2011
Registered Attendee November 2010

Society of Forensic Toxicologists (SOFT)

Student Enrichment Program (SEP) Participant July 2012