MITRAGYNA SPECIOSA: AN ANALYTICAL STUDY

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ABSTRACT

Legal highs are compounds that produce the same psychoactive effects as illicit compounds, but are not legally controlled under the Controlled Substances Act. While Kratom has been used for centuries by Thai laborers, it has recently been offered as a legal high by online markets and local head shops. Kratom is an indigenous plant to Southeast Asia, and the botanical name is *Mitragyna speciosa*. It is a mild stimulant, and the traditional use is to chew on leaves to overcome fatigue.

Kratom is unique in that it is capable of producing both stimulant and sedative effects depending on the dose administered. At low doses, kratom produces effects similar to a stimulant. Users report feeling increased alertness, physical energy, talkativeness, and sociable behaviors. At high doses, kratom produces effects similar to opiates and sedatives as well as euphoric effects.

The objective of this project was to develop a Gas Chromatography-Mass Spectrometry (GC-MS) method to detect mitragynine and 7-hydroxymitragynine in kratom for adoption by forensic crime labs. Seven powdered samples, one capsule, four liquid samples, and two different varieties of pills were obtained for analysis. Mitragynine has been detected in all of the samples except for the two pill samples; however, these two samples were not marketed as kratom, nor did they list mitragynine or *Mitragyna speciosa* as an ingredient. The 7-hydroxymitragynine has not been detected in any of the samples tested.

Keywords: legal highs, kratom, mitragynine, gas chromatography-mass spectrometry, GC-MS, 7-hydroxymitragynine

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The objective of this project was to develop a GC-MS method to detect mitragynine and 7-hydroxymitragynine in kratom samples that could be adopted by forensic crime labs. While psychoactive properties of kratom have been known for centuries, it has recently become available in head shops and from online vendors. Although kratom is not currently controlled at the federal level, several states have added it to their controlled substances lists[1], and the Drug Enforcement Agency (DEA) has listed it as a drug of interest.[2] One of the most interesting and fastest growing issues in forensic science is the analysis and identification of legal highs. Since 2008, legal highs have grown in popularity because they produce effects similar to those of illicit drugs, but are not themselves legally controlled. These drugs are most often produced by taking the structure of an illicit compound and then slightly altering its structure to create a compound that produces effects similar to the illicit drug but is structurally different enough so that it is not illegal to possess. One of the original drugs marketed as a bath salt was 3,4-methylenedioxypyrovalerone (MDPV) which was formed by the addition of a methylenedioxy group to the Schedule V drug pyrovalerone.[3] Figure 1 shows the structure of both of these compounds.

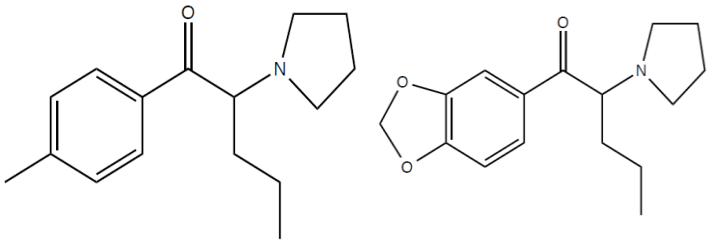

Figure 1 Structure of Pyrovalerone (left) and MDPV (right)

Even though compounds can be structurally changed to imitate the effects of an illicit compound, addicts may also turn to plants that have psychoactive properties to obtain similar highs. One such plant that is increasing in popularity in the United States is kratom.

Botanical Origin and History

Historically, kratom has many names including *biak-biak* or *Ketum* in Malaysia and *Kakaum, Kraton, Ithang,* and *Thom* in Thailand, but the botanical name is *Mitragyna speciosa*.[4] Kratom is native to Southeast Asia, and is in the *Rubiaceae* family along with the coffee tree and gardenia plants.[5] It is a tree that can reach heights of up to fifty feet and a spread of more than fifteen feet wide. The leaves are dark green in color and have an ovate shape with a tapered end. The flowers are yellow and grow in clusters, and the seeds are winged. Leaves are shed and continuously replaced throughout the year.[4]

The traditional use of kratom leaves is as a mild stimulant.[6] Thai laborers and farmers chewed on leaves to overcome effects of working under prolonged sun exposure. The first medicinal use of kratom can be traced back to Malaysia in 1836, when it was used as an opium substitute. Kratom has also been used as treatment for intestinal

infections, diarrhea, muscle pain, and coughing.[4] A more recent trend in kratom use is as a self-treatment option for opioid withdrawal symptoms. Kratom is attractive to users because of the low price of $10 to $40 per 28 grams of plant material, with a suggested dose of one to eight grams.[7]

Even though all kratom is from the *Mitragyna speciosa* species, some studies have indicated the possibility of variation among the chemical constituents of differing batches of leaves, indicating the presence of geographical variants of species within Thailand. In the study conducted by Takayama et al., 66.2% of the crude extract from the plants grown in Thailand was mitragynine.[8] Speciogynine accounted for 6.6%, speciociliatine was 0.8%, paynantheine was 8.6%, and 7-hydroxymitragynine constituted 2.0%.[8] These five compounds were also found in the Malaysian plant, but at lower concentrations. Only 12% of the Malaysian extract was mitragynine. Since forensic scientists may not know the levels of compounds present in the samples they receive, it is important that the method used be able to detect mitragynine and/or 7-hydroxymitragynine at low levels so that samples can be properly identified.

Routes of Ingestion and Perception

One of the most popular routes of administration is brewing the leaves into a tea. Another popular route is simply chewing on a couple leaves at a time. Users also report smoking dried leaves, or making them into an extract.[4] Lemon juice is often used when making extracts, because the acidity of the lemon juice helps the alkaloids extract into water. Some users will also mix the plant material into a beverage such as a milk shake,

juice, or soda. In order to mask the bitter taste of the plant, some users will add sugar or honey to the cocktail. Other users claim that the addition of salt to the fresh leaves helps to prevent constipation.[9] A popular practice in Malaysia is to create "madatin." These are pills made by boiling dried kratom leaves in water to create syrup. This syrup is then mixed with finely chopped leaves from the palas palm to create "madatin" which is then smoked in bamboo pipes.[4] Another popular practice is to chew fresh leaves with betel nuts. A beverage rising in popularity in Southern Thailand, known as 4x100, contains kratom leaves as one of four ingredients. The other three ingredients are a caffeinated soft drink, a cough syrup containing codeine or diphenhydramine, and an anxiolytic, antidepressant, or analgesic drug. This cocktail has potentially fatal consequences due to the unknown multidrug interactions.

Assanangkornchai et al. conducted a two-phase survey of individuals in Thailand who were kratom users, as well as individuals who were acquainted with kratom users. The purpose of the study was to gauge the public opinion of kratom and to examine the patterns of use. The first phase surveyed 47 known kratom users and 19 non-using family members, neighbors, and friends.[6] The second phase surveyed 433 individuals who were divided into three categories: long-term, regular users (149), occasional users (168), and nonusers who lived in the same village (116). Approximately 84% of the individuals polled believed that the main reason for using kratom was to increase work efficiency. Eighty percent of long-term users stated it gave them better tolerance to being exposed to the hot sun. Roughly 61% of polled individuals agreed with each other that kratom use is a private and personal issue, and 64% of polled individuals agreed with each other that users were not disturbing anyone else by using kratom. Regular users were twice as likely

as non-users to have family members who were kratom users. Most users (69%) first tried kratom with a family member or friend, and the most common motivation was the desire to experience the effects. Of everyone surveyed, 61% of regular users and 12% of occasional users believed that they were dependent on kratom. They cited their dependence based on symptoms such as the inability to quit using kratom and a strong desire for kratom when not using it. Users also cited a physical pain in their back, legs, or muscles that was caused by cessation of kratom ingestion.

Increasing Popularity

The prevalence of kratom in the West is increasing. The System to Retrieve Information from Drug Evidence (STRIDE) and the National Forensic Laboratory Information System (NFLIS) reported one case of mitragynine in 2010, 44 reports in 2011, and 81 reports in the first six months of 2012.[10] Kratom was also placed on the DEA List of Chemicals of Concern in 2010. A search in 2012 of the US National Library of Medicine's PubMed database resulted in 30 hits on the term "kratom" since 2004.[5] This is compared to four articles published between 1988 and 1997, and one article in 1975. Another PubMed search using "*Mitragyna speciosa*" as the keyword resulted in 65 published articles, 49 of which were published after 2004. These same searches today result in 62 and 99 hits, respectively. A Google search of "kratom" resulted in over 700,000 hits. Boyer et al. conducted a study of users on www.drugbuyers.com who indicated purchasing kratom for use of managing opioid withdrawal symptoms.[7] They tracked the number of times kratom was mentioned from November 2004 to October

2005. Beginning in April 2005, there was a huge increase in kratom mentions, from less than 25 to over 400 mentions by the end of October. This trend coincided with the release of the 2005 U.S. National Drug Intelligence Center Report that described treatment of opioid withdrawal symptoms as one of the potential applications of kratom. Boyer et al. categorized kratom mentions into five categories: 1) use during opioid holidays, 2) as an opioid replacement therapy, 3) opioid analgesic agents and in the context of previous illicit drug use, 4) as an economical alternative to opioid analgesics, and 5) as providing hope to opioid-tolerant persons. Opioid holidays are when users intentionally stop taking opioids for a temporary amount of time in an effort to reduce their tolerance as well as reduce the cost of treatments when they begin using again.

Legal Status and Effects

The legal status of kratom varies from country to country. In 1979, Thailand enacted the Addictive Substances Law, which placed kratom in Category V along with drugs such as cannabis, opium, and hallucinogenic mushrooms.[10] In Malaysia, mitragynine was listed in January 2003 in the First and Third Schedules as a psychotropic substance of the Poisons Act of 1952; however, planting the kratom tree is not considered an offense and local law enforcement have no authority to remove the trees.[11] In 2005, the maximum penalty for an offense was a fine of 10,000 ringgit (equivalent to about $2,800) and/or a 4-year jail sentence.[11] Kratom is illegal in Australia, New Zealand, Romania, Russia, Israel, Denmark, Myanmar, and South Korea.[9] It is a controlled medicine in Finland and requires a prescription to purchase. Kratom is currently not

scheduled under the Controlled Substances Act in the United States. A few states such as Indiana, Louisiana, and Tennessee have passed laws to make the possession or sale of kratom, or possession of the active ingredients in Kratom, illegal.[9] Alabama recently passed a law that lists mitragynine as a Schedule I compound. In 2010, the DEA's Drugs of Chemicals of Concern list stated that there is no current accepted medical use for kratom in the United States. The United States Food and Drug Administration (FDA) issued import alert 54-15 in February 2015 that allows for the detention, without physical inspection, of dietary supplements and bulk dietary ingredients that contain *Mitragyna speciosa* or kratom.[12]

The effects of kratom vary greatly with dose. A low dose is 1-5 grams of dried leaves, a moderate dose is 5-15 grams, and 16 grams or more constitutes a high dose.[5] Kratom is unique in that it is capable of producing both stimulant and sedative effects, depending on the dose administered.[10] At low doses, kratom produces effects similar to a stimulant. Kratom users report feelings of increased alertness, physical energy, talkativeness, and sociable behavior.[10] Some users report that these doses can also cause an unpleasant sense of anxiety and internal agitation. Moderate doses produce effects similar to opiates such as analgesia, constipation, euphoria, and sedation. The euphoric effects tend to be less potent than those produced by opium. Very high doses cause sedative effects and can also induce stupor, mimicking opioid effects.[5] Effects can occur within five to ten minutes of ingestion and last anywhere from two to five hours. Acute side effects include nausea, itching, sweating, dry mouth, constipation, increased urination, and loss of appetite. Long-term use side effects are anorexia, weight loss, insomnia, skin darkening, dry mouth, frequent urination, and constipation. Withdrawal

symptoms experienced by some individuals include hostility, aggression, extreme mood swings, wet nose, aching muscles and bones, and jerky movements of the limbs.[10] There is a report indicating the successful use of dihydrocodeine and lofexidine to manage an individual's kratom withdrawal symptoms.[5] Several cases of psychosis caused by kratom have also been reported. Addicts have exhibited psychosis symptoms including hallucinations, delusion, and confusion.[10]

Erowid User Reports

Erowid is a popular online drug forum in which users can search for information on the history of a drug and legal status of a drug, but they can also add self-experience reports on taking a drug.[13] A brief search yielded 247 results for experiences with kratom, but only one experience with 7-hydroxymitragynine. The user posting the 7-hydroxymitragynine experience stated that effects were felt within a minute and were very similar to the high of hydrocodone.[14] The interesting aspect of this experience is that the user had suffered an injury in his left elbow that left residual nerve pain, and after the administration of 7-hydroxymitragynine the pain disappeared and even stayed gone after the euphoric effects of the 7-hydroxymitragynine subsided. The user reported that while taking other opiates the pain was always present, it just was not as debilitating. Kratom user experiences are divided into several categories including: general, first times, combinations, retrospective/summary, preparation/recipes, difficult experiences, bad trips, health problems, train wrecks and trip disasters, addiction and habituation, glowing experiences, mystical experiences, health benefits, and medical use.[15]

The user experiences of kratom include both positive and negative accounts. In one positive account, the user prepared a kratom tea with six grams of extract.[16] Forty-five minutes after ingestion, the user experienced minor visual hallucinations while staring at the floor, such as the floor moving and waving. For the next two hours, the user reported feeling "the most fantastic high…similar to an orgasm crossed with a marijuana high." The user also experienced shivers of warmth running the length of his body and his fingers while other extremities were numb. Any movement caused warm tingling sensations that were especially noticeable around his head and neck area, and a touch from another person caused a sensational feeling. A different user ingested a small four gram vial purchased from the internet, and likened the experience to the high of mushrooms without the elaborate visuals.[17] The user reported that half an hour after ingestion, he felt as though the spirit of kratom was nurturing, warming, and caring about him. He was able to visualize himself in an unbiased light and realized the negative aspects of his personality. He spent the next hour in a pleasurable state experiencing a self-healing process. In a negative account, the user prepared a tea using only two teaspoons of kratom.[18] She states that it had a bitter taste, but was similar to other herbal teas she's had. Immediately after ingestion, she began to feel a slight buzz similar to the buzz felt from alcohol. Shortly after the buzz, she began to feel nauseous and the sensation grew stronger until she was covered in sweat and dry-heaving. She went to bed, and in the morning she still felt weak and nauseous. Attempting to drink water and juice caused violet stomach cramps and vomiting. Another user ingested 23 grams as a tea, and 15 minutes after ingestion he felt his heart rate increase greatly.[19] After a half hour with an increased heart rate he chose to lie on the couch, and began to feel waves of nausea.

When he closed his eyes to cope with the nausea, he began to hallucinate. Around an hour and a half after ingestion, he felt extremely nauseous and spent two hours in the bathroom vomiting and heaving. The user ingested the tea at 10 pm and was incapacitated from nausea until 3 pm of the following day.

Pharmacology

The proposed active alkaloids of kratom are mitragynine and 7-hydroxymitragynine.[8] The major constituent is mitragynine, and 7-hydroxymitragynine is a minor constituent. The structure of these two compounds can be seen in Figure 2.

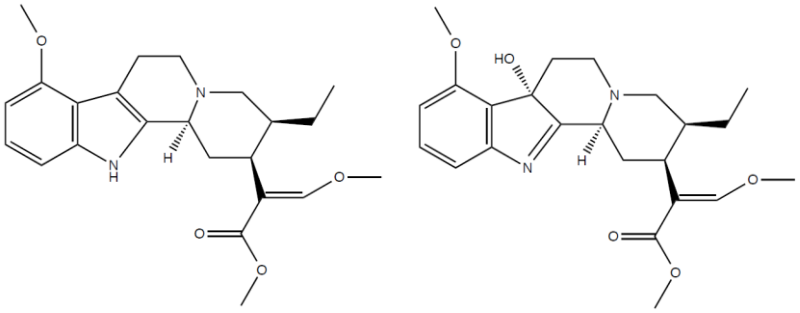

Figure 2 Structure of Mitragynine (left) and 7-Hydroxymitragynine (right)

Three other minor constituents, speciociliatine, speciogynine, and paynantheine, have been shown to be potential contributors to the effects produced by kratom. Unlike mitragynine and 7-hydroxymitragynine, however, these compounds are not inhibited by the opioid antagonist naloxone, suggesting that their mechanism of action is independent of the opioid receptors.[5] Mitragynine has been reported to be 26% as effective as

morphine as an opioid agonist, while 7-hydroxymitragynine is reported to be 1,071% more effective as an opioid agonist than morphine.[8] It was reported that 7-hydroxymitragynine displayed a 13-fold higher potency than morphine in the guinea pig ileum contraction test.[20]

Mitragynine displayed *in vitro* activity at both supraspinal opioid mu and delta receptors, while 7-hydroxymitragynine reacted with all three opioid receptors, but had the greatest affinity for the mu receptors, with an affinity of 89.9%.[21] The affinities for the delta and kappa receptors were 5.6% and 4.6% respectively.[20] The mu receptors are responsible for mediating analgesia, euphoria, and respiratory depression. The activity of mitragynine and 7-hydroxymitragynine on these receptors explains the analgesic effects of kratom, as well as the effectiveness of kratom when used to treat opioid withdrawal symptoms. It was determined that the methoxyl group at the C9 position is essential for the analgesic activity of mitragynine. This determination was made by the observation that corynantheidine lacks opioid activity. The only structural difference between these two compounds is the methoxyl group at C9 being present in mitragynine and absent in corynantheidine.[8]

When administered subcutaneously to mice, the maximum possible effect (MPE) value for 7-hydroxymitragynine reached 100% within 15-30 minutes after administration, whereas morphine's MPE value was at 69% 45 minutes after administration. A similar difference was noted with the hot plate test. A hot plate test is a common test of pain response in animals, and is conducted by placing an animal on a hot plate and measuring the time it takes for the animal to show a response to the heat such as licking a paw or jumping. The MPE for 7-hydroxymitragynine peaked at 94% after 15 minutes, whereas

morphine's MPE peaked at 79% at 30 minutes.[20] When administered orally to mice, 7-hydroxymitragynine possessed potent antinociceptive effects in tail-flick and hot-plate tests. In the tail flick test, the MPE value of 7-hydroxymitragynine reached 100% in 15-30 minutes and a potent antinociceptive effect lasted for 90 minutes. The MPE value of morphine was 49% at 45 minutes. In the hot plate test, 7-hydroxymitragynine's MPE value reached 87% in 15 minutes. These results indicate that 7-hydroxymitragynine is readily absorbed when administered orally, whereas the liver rapidly metabolizes an oral dose of morphine and excretes the morphine in urine.[20]

Toxicity

The toxicity of mitragynine is relatively low. Animal studies have been conducted using doses as high as 920 mg/kg administered to dogs, and no toxicity, measured in the form of tremors or convulsions, was reported.[9] A more recent study, however, reported lethal toxicity when a dose of 200 mg of pure mitragynine was administered orally to rats. When both mitragynine and 7-hydroxymitragynine are given to animals for five days or longer, both produce physical dependence and withdrawal symptoms similar to those of opioid withdrawals.[5]

There have also been reports of toxicity of kratom in humans.[4] In 2008, a 32-year-old male had a seizure caused by an overdose of kratom. He developed fever and aspiration pneumonia after the cessation of the seizure. Similarly, a 64-year-old male had a seizure after consumption of kratom and a toxicology report confirmed the concentration of mitragynine in his system to be 167 ± 15 ng/mL. A 44-year-old male

developed severe primary hypothyroidism after four months of heavy kratom use. A male in Germany developed jaundice and itching after taking overdoses of kratom powder over the course of two weeks. A 43-year-old white male with a medical history of chronic pain was admitted for evaluation because of a tonic-clonic seizure. He was self-treating by injecting 10 mg of hydromorphone subcutaneously daily. When hydromorphone was unavailable, he used kratom to mediate his opioid withdrawal symptoms. He spent $15,000 a year on kratom and drank it as a tea four times a day. He indicated substantial pain relief, as well as increased alertness without the drowsiness associated with opioid use. Attempting to increase alertness, he took 100 mg of modafinil with kratom. Twenty minutes after ingestion, he experienced a generalized tonic-clonic seizure lasting five minutes. After the seizure, he quit using kratom and experienced withdrawal lasting 10 days. His symptoms included rhinorrhea, insomnia, poor concentration, constricted affect, and myalgia.[22]

Several reports of toxicity stemmed from krypton, which was a very potent form of kratom sold in Sweden. During a five-year period, there were nine reports of deaths related to krypton use. Krypton contained mitragynine and *O*-desmethyltramadol, and it was speculated that an interaction between the two was causing the deaths.[5] Another recent report of death in the U.S. was associated with mitragynine and 7-hydroxymitragynine and propylexedrine use. The case for toxicity of kratom in humans is not overwhelming, however, there is growing evidence that kratom should be taken seriously by emergency medical personnel and law enforcement. A hazardous possibility is highly concentrated mitragynine and 7-hydroxymitragynine being mixed with other

psychoactive drugs such as alcohol, sedatives, and cannabinoids and producing very serious health risks.[5]

Analytical Methods

Kikura-Hanajiri et al. developed a method for the simultaneous analysis of mitragynine and 7-hydroxymitragynine in kratom via liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). Mitragynine and 7-hydroxymitragynine were detected in eleven of the thirteen samples analyzed, with the concentration of mitragynine ranging from 1% to 6% and 7-hydroxymitragynine ranging from 0.01% to 0.04%.[23] Of the thirteen samples analyzed, six were in the form of dried leaves, four were in the form of powders, and three were in the form of resins. Ten to fifty milligrams of each sample were used in the extraction process. Samples were ground into a fine powder, extracted with 10 mL of 80% methanol, and exposed to ultrasonication for an hour. Samples were stored at room temperature overnight and then centrifuged and filtered before injection into the LC. The concentration of mitragynine and 7-hydroxymitragynine was greater in the resin samples than any of the other samples. The two samples that provided negative results were both dried leaf samples, and it was noted that the shapes of the leaves in these two products were different than the samples that were positive. One negative sample did have a small concentration of mitragynine, but not 7-hydroxymitragynine, whereas the other sample was negative for both compounds.

Chan et al developed a GC-MS method to identify mitragynine in kratom samples; however, there was no attempt to identify 7-hydroxymitragynine. The focus was

on analyzing leaves, powdered leaves, and liquid samples such as drinks and teas. Two grams of leaves or powdered leaves were ultrasonicated in 25 mL of a 1:4 mixture of $CHCl_3$:MeOH for 10 minutes. The solution was allowed to settle, and then an aliquot was taken for analysis.[11] Liquid samples were acidified with a few drops of concentrated HCl and then extracted with 20 mL of diethyl ether. The aqueous layer was removed, basified with NaOH, and then extracted twice with 20 mL of chloroform. The combined extracts were washed with water and then filtered through anhydrous sodium sulphate and left to dry in a fume hood. The extract was then dissolved in one milliliter of methanol for analysis by GC-MS. The temperature program used was 200 °C held for two minutes with a ramp of 10 °C per minute to 300 °C with a final hold for 20 minutes. Mitragynine was detected in all of the analyzed samples.

Philipp et al. created a GC-MS method to identify mitragynine, paynantheine, speciogynine, and speciociliatine in urine through derivatization with TMS, enzymatic cleavage of conjugate, and solid-phase extraction. The proposed procedure resulted in a limit of detection of 100 ng/mL. The oven temperature program started at 80 °C with an initial hold of two minutes followed by a 30 °C/min ramp to 310 °C with a final hold of five minutes for a run time of 14.67 minutes. One hundred and twenty human urine samples were analyzed. Of the 120 urine samples tested, 98 were positive for mitragynine. A few samples also tested positive for speciogynine, speciociliatine, and paynantheine. It should be noted that the samples received for analysis were submitted anonymously and did not contain information about how the kratom was ingested, the dose, the time interval from administration, or the form of kratom that was taken.

Sanagi et al. developed a method to identify mitragynine in *Mitragyna speciosa* and a similar species by utilizing an ultrasonic-assisted extraction procedure coupled with gas chromatography mass spectrometry.[24] The leaves (4.0 g) were crushed and soaked in 25 milliliters of a 1:4 chloroform:methanol solution. The solution was ultrasonicated at 30 °C for one hour and then stored overnight at room temperature. The extract was then filtered through a 0.45 μm Whatman nylon membrane filter into a clean, glass tube and evaporated under a vacuum at 30 °C for 45 minutes. An aliquot of one microliter of the reduced extract was injected onto the GC-MS. The oven program had an initial temperature of 70 °C with a hold of two minutes. The temperature was ramped at 7.5 °C/min to 280 °C, with a final hold of 15 minutes for a total analysis time of 45 minutes. Sanagi et al. were able to detect mitragynine in the two *Mitragyna speciosa* samples that were analyzed; however, mitragynine was not detected in the sample of the other species.

Wang et al. compared three instrumental techniques for the analysis of mitragynine and other alkaloids in *Mitragyna speciosa* samples.[25] The three techniques were gas chromatography with mass spectrometry, supercritical fluid chromatography with diode array detection, and ultra-high-pressure liquid chromatography with mass spectrometry. A standard solution of eight alkaloids found in kratom (corynoxine B, corynoxine, paynantheine, 3-isopaynantheine, mitragynine, speciogynine, speciociliatine, and 7-hydroxymitragynine) was prepared and run on each of three instruments.

The GC-MS oven temperature program began with an initial temperature hold of two minutes at 220 °C followed by an increase of 8 °C per minute to 300 °C with a final hold at 300 °C for 10 minutes. This produced a total analysis time of 22 minutes. The extraction procedure for the experimental samples used by Wang et al. required 550

grams of *Mitragyna speciosa* leaves to be extracted in three liters of methanol for twenty-four hours.[25] This process was repeated four times. The solvent was then removed under reduced pressure to yield a dried extract. An aliquot of the extract was reconstituted in 5% HCl in water and extracted with ethyl acetate. The aqueous layer was basified with liquid ammonia and extracted again with ethyl acetate. The organic layer was then separated and dried under reduced pressure producing an alkaloid extract. All eight compounds prepared in the standard mixture were detected in the alkaloid extract. Two pairs of diastereoisomers, Corynoxine and corynoxine B, and mitragynine and speciociliatine, were not resolvable using GC-MS.

Wang et al. provide two cautions for utilizing GC-MS as an analytical technique for kratom samples, the high temperature required to elute the alkaloids and the inadequate separation between mitragynine and speciociliatine.[25] The only difference between mitragynine and speciociliatine is the orientation of the hydrogen at the 3-position. It would be possible, however, to differentiate between the two compounds by comparing the difference in abundance between the molecular ion 397 and the 383 ion, which is produced by the loss of a methyl group. The ratio of the 397/383 ions is greater than one in mitragynine, whereas the ratio in speciociliatine is less than one. The required high temperatures are cited as a caution because they are close to the upper temperature limits of the gas chromatography column, and therefore there is not a lot of room for temperature adjustments in an effort to increase compound mixture resolution.

Lesiak et al. used direct analysis in real time coupled with mass spectrometry (DART-MS) to analyze kratom samples.[26] Four kratom plants of different varieties were purchased for analysis by DART-MS. All plants were six to seven months old. Fresh

leaves were sampled by taking small clippings of the plants and holding them with tweezers in-between the ion source and the mass spectrometer inlet. Extracts of the leaves were prepared by soaking each leaf clipping in 50 μL of absolute ethanol and sonicating for 20 minutes. The sample was then allowed to stand for two hours before analysis. Four microliter aliquots of each extract were then placed in sampling windows on a 12 sample QuickStrip^{TM} sampling card, which allowed for greater sample throughput. It also allowed for consistent positioning of samples in the helium stream as the sampling card was passed through it at a rate of one millimeter per second. Mitragynine, paynantheine, 7-hydroxymitragynine, and corynoxine were detected in all of the analyzed samples. This method shows promise for quick kratom identification as 250 leaf samples could be analyzed in an hour by this method. Since mitragynine and 7-hydroxymitragynine are unique to kratom, the presence of these two compounds can act as confirmation of the sample coming from a kratom plant. It was noted that testing the leaf clippings directly yielded more information than the extract. Over 140 compounds were identified in the DART-MS spectrum of one of the plant clippings, whereas only 62 compounds were identified in the ethanol extract of that clipping. This reduction of obtained information is roughly 56%; however, the extract did yield enough information that the sample would be able to be identified as kratom.

Gas Chromatography and Mass Spectrometry

Chromatography is the combination of the Greek words *chroma*, meaning "color" and *graphein* meaning "writing."[27] The term chromatography can be attributed to the

inventor of the technique, Mikhail Tswett, who separated the pigments found in plants (chlorophylls and xanthophylls) creating colored bands in the column. Chromatography is a term encompassing techniques focused on separating individual components of a mixture. This separation is achieved by differential separation, which is caused by various degrees of interaction of compounds with a stationary phase as they are pushed through a column by the mobile phase.[28] The process begins with the sample injection. The sample is injected into a heated inlet, which vaporizes the sample before it is introduced into the column. There are three types of sample injection: split, splitless, and on-column. A split injection is when only 0.1-10% of the injected sample is introduced to the column, and the rest is disposed of as waste. This type of injection is most often used in routine methods. Splitless injections occur when 80% or greater of the sample are introduced to the column. This type of injection is useful for quantitative analysis, as well as for analysis of trace components of a mixture. The final type of injection is on-column injection. This is where the sample is introduced directly into the column. This technique is best used to analyze compounds that decompose when subjected to temperature greater than their boiling temperature. The sample is pushed through the column by the mobile phase, or carrier gas. In gas chromatography, the mobile phase is an inert gas such as argon, helium, nitrogen, and hydrogen, with helium being the most common gas used.[29] The stationary phase for gas chromatography is present in the column. There are three types of columns commonly used: wall-coated open tubular column (WCOT), support-coated open tubular column (SCOT), and porous-layer open tubular column (PLOT). The difference between these columns is how the stationary phase is located on the inside of the column. A WCOT column has a stationary liquid phase coated on the inside of a

column. A SCOT column has a layer of solid particles on the inside of the column, which is coated with a stationary liquid phase. A PLOT column has a layer of stationary solid-phase particles coating the inside of the column. The column used in this research is a DB-5 column, meaning that the stationary phase is composed of 95% dimethylpolysiloxane and 5% phenyl-methylpolysiloxane. The column is located in an oven, and the temperature of the oven is controlled and can be changed during a run. Changing the temperature during the analysis of a sample can help separate compounds that have a wide range of boiling points and/or polarities. Raising the column temperature can also decrease the retention time of compounds, thereby shortening the total analysis time, and sharpen the peaks for better resolution between compounds.

After compounds have traveled the length of the column they are introduced to the detector, or mass spectrometer. A mass spectrometer measures the masses and abundances of ions in the gas phase. It is useful because it is sensitive enough to detect low concentrations of an analyte, provides both qualitative and quantitative data, and can differentiate between compounds eluting from the gas chromatograph at the same time.[30] Before compounds can be detected by the mass spectrometer, they must be converted into ions. This conversion occurs in the ionization chamber and there are two common methods for ionization: electron and chemical. Electron ionization occurs when a compound encounters a stream of electrons that bombard the compound. Some analyte molecules absorb enough energy to ionize, and this energy causes compounds to fragment in characteristic ways. Chemical ionization is a softer ionization method than electron ionization and occurs when energized electrons convert a reagent gas, such as methane, to several species. Methanium (CH_5^+) is formed from this process and reacts

with analyte to form protonated molecules because of its potent proton donating ability. After ionization, the sample is introduced into the quadrupole mass separator. This section is composed of four parallel rods, which have both a constant voltage and oscillating frequency voltage applied to them. These changing voltages create an electric field that only allows ions with a certain mass to charge ratio to reach the detector. Other ions are deflected and collide with the rods, causing them to be lost. The voltages can be constantly changed to allow ions with different masses to reach the detector.

MATERIALS AND METHODS

Materials

Fourteen samples were purchased for this experiment. Eleven of the samples were purchased from Amazon.com. They were found by typing in “kratom” in the Amazon.com search bar. One sample, O.P.M.S. Liquid Kratom, was purchased from the local head shop, Cloud Nine. Two of the three VivaZen samples were purchased from a local convenience store. Standards of mitragynine and 7-hydroxymitragynine were purchased from Cerilliant (Round Rock, TX). The mitragynine standard was at a concentration of 100 μg/mL in methanol, and the 7-hydroxymitragynine standard was at a concentration of 100 μg/mL in 0.1 N ammonia in methanol.[31,32] Dibucaine from Fisher Scientific was selected as the internal standard and was prepared in chloroform at a final concentration of 0.1 mg/mL.

Methods

Limit of Detection

A serial dilution was prepared from the mitragynine 100 μg/mL standard by pipetting 250 μL of the standard into a GC vial, along with 250 μL of chloroform for a total volume of 500 μL. Fifty microliters of a dibucaine solution were also added to this sample for a new total volume of 550 μL. The mitragynine concentration in this standard

was 45.5 μg/mL. This process was repeated to prepare three more samples with a mitragynine concentration of 20.7 μg/mL, 9.41 μg/mL and 4.28 μg/mL. Each sample was run on the GC three times, resulting in 15 data files. To calculate the limit of detection, the sample chromatograms were opened in the Mestrenova software (Mestrelab Research, Santiago de Compostela, Spain), and expanded to show only the range of 20-28 minutes of the run. The peak height and height of the baseline near the peak were measured. The baseline was measured by drawing a line at the highest point, and another line at the lowest point. The distance between these two lines was used as the noise (a). The peak height was measured by drawing a line at the top of the peak, and a line through the middle of the noise at the baseline of the peak. The distance between these two lines was used as the height (H). The formula $H \geq 3a$ was used to determine if the mitragynine peak could reliably be identified. If the peak height (H) was not larger than three times the noise (a), then mitragynine could not be detected at that concentration. The lowest concentration where mitragynine could be detected was set as the limit of detection.

Reproducibility

The same stock mitragynine standard was run ten times on different days and at different times of day to document the reproducibility of the method.

Instrumentation and Sample Preparation

The samples were run on an Agilent Technologies Gas Chromatography/Mass Spectrometer with a 6890N Network GC System and a 5975 Inert Mass Selection Detector. The instrument was equipped with a 7683B Series Injector and a 7683 Series Autosampler. The column was a DB-5 column (30 m x 0.25 nm, with 0.25 μm film thickness). Helium was used as the carrier gas with a constant flow rate of 1.1 mL/min. The solvent delay was set at four minutes. A split injection was used with a split ratio of 15:1 and an injection volume of 2.0 μL. The injection port temperature was set at 260 °C. The oven temperature program was as follows: initial temperature of 150 °C held for two minutes, then a ramp of 20 °C/min to 275 °C with a final hold of 20 minutes for a total run time of 28.25 minutes. All spectra were analyzed using ChemStation software. Peaks tentatively identified as mitragynine were compared against the NIST05 mass spectra library, as well as against the spectrum obtained from the purchased standard. The dibucaine peaks were also compared against the NIST05 mass spectra library. Other peaks present in the spectra were compared to literature values as well as the NIST05 library in order to presumptively identify other compounds present in the kratom samples.

The eight powdered samples were prepared for analysis by measuring ~0.5 grams of the sample and adding it to 15 mL of chloroform in a 25 mL Erlenmeyer flask. The flask was then placed in a sonicator and sonicated for 30 minutes. After sonication, the sample was filtered into a beaker using a Buchner funnel with Whatman Number 4 filter paper. The sample was placed on a hot plate and gently heated down to a small volume (~2 mL) to increase the concentration of mitragynine and other compounds extracted

from the sample. The sample was then pipetted into a GC vial and 50 µL of a dibucaine solution were added and the sample was analyzed by GC-MS. The capsule sample was analyzed in a similar manner, with the exception that the entire contents of the capsule were used for analysis since the powder inside the capsule weighed 0.4096 grams. The two samples in pill form were prepared for analysis using the same method, with the exception that the pill was crushed using a mortar and pestle before being weighed out.

The four liquid samples were prepared for analysis using a basic drug extraction procedure. Three milliliters of the sample were placed in a test tube and acidified with 10 drops of 0.1M HCl to pH 1. Three milliliters of chloroform were then added and the solution was mixed. The chloroform layer was removed, placed in a GC vial and analyzed by GC-MS. The pH of the remaining aqueous layer was raised to 10 by adding five drops of 4M NaOH. Three milliliters of chloroform were added and the solution was mixed. The chloroform layer was removed, placed in a GC vial and analyzed by GC-MS.

RESULTS AND DISCUSSION

Method Development

The GC method developed in this project is based on the one provided by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) kratom monograph.[33] The run time of the SWGDRUG method was 41.3 minutes, which was shortened to 28.3 minutes in this project by adjusting the oven temperature parameters. The SWGDRUG oven program used an initial temperature of 100 °C and a ramp rate of 14 °C/min up to 300 °C. In this experiment the initial temperature was 150 °C with a ramp rate of 20 °C/min to 275 °C. The final hold in the SWGDRUG method was 25 minutes, whereas the final hold in this method was 20 minutes.

In an effort to be consistent with forensic labs that require short extraction times, sonication of the sample for 30 minutes in chloroform was sufficient for extraction and detection of mitragynine in the experimental samples.

Dibucaine was selected as the internal standard (ISD) because it is commonly used as the internal standard at the Alabama Department of Forensic Sciences. Even though the retention time is not close to that of mitragynine or other alkaloids found in kratom, using dibucaine as the ISD will make the method more easily adapted in Alabama.

Table 1 shows peaks present in the mass spectra of mitragynine peaks from three sources: the standard purchased from Cerilliant[31], a spectrum produced by the United

Nations Office on Drugs and Crime (UNODC)[24], and one of the samples analyzed in this experiment. Table 1 shows the similarities between the mass spectra of these samples, which were used for the identification of mitragynine in the experimental samples. The *m/z* of 214, 397, and 383 were selected as criteria for identifying mitragynine.

Table 1 Mass Spectra Peak Comparison of Cerilliant Standard, UNODC Sample, and Experimental Sample

Cerilliant Standard	UNODC Sample	Experimental Sample
91	91	91
186	186	186
214	214	214
239	239	239
269	269	269
311	311	311
327	327	327
367	367	367
383	383	383
397	397	397
429	429	429

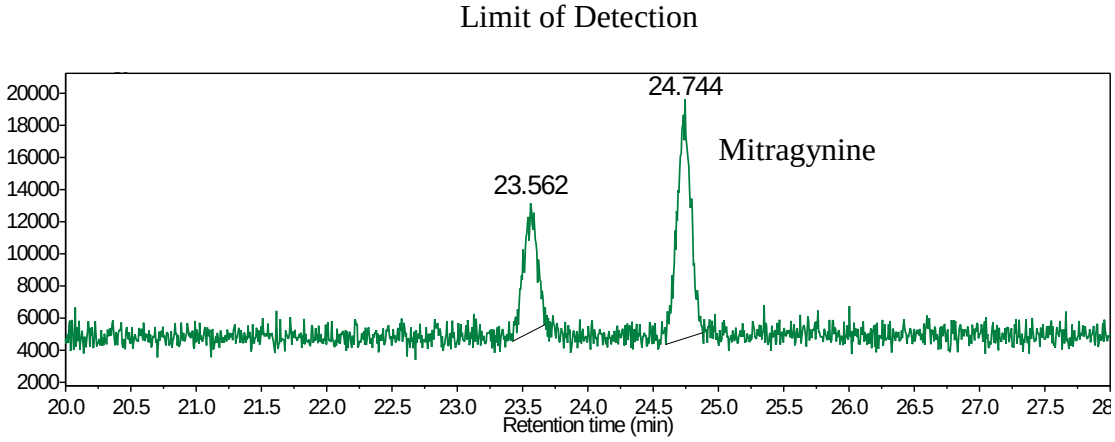

Figure 3 Mestrenova Chromatogram of 9.41 µg/mL Sample

An example of the expanded chromatogram of a 9.41 μg/mL sample created in the Mestrenova software (Mestrelab Research, Santiago de Compostela, Spain) is shown above in Figure 3. In order to be considered a valid peak, its height has to be three times greater than the height of the noise in the baseline. The calculated peak heights, noise, and equation used can be seen in Table 2.

Table 2 Calculated Peak Heights, Noise, and Equation Used for the Limit of Detection

Sample	Peak Height, (H)/cm	Noise, (a)/cm	H≥3a
4.28 μg/mL	3.9	2.2	~~3.9≥6.6~~
9.41 μg/mL (1)	4.5	1.1	4.5≥3.3
9.41 μg/mL (2)	10.4	2.4	10.4≥7.2
9.41 μg/mL (3)	7.4	1.6	7.4≥4.8

The mitragynine standard (100 μg/mL) received from Cerilliant was the highest available concentration used in the limit of detection study. At a concentration of 4.28 μg/mL, mitragynine was no longer reproducibly detectable or discernable from base noise. The first 4.28 μg/mL sample analyzed for the limit of detection had an "H" value of 3.9 cm and an "a" value of 2.2. When these values were plugged into the formula stated above, the value of "H" was less than that of "3a" (3.9≧6.6). Therefore, mitragynine peaks at a concentration of 4.28 μg/mL are not reproducibly discernable from the baseline noise and this standard concentration could not be used as the lower limit of detection. The three 9.41 μg/mL samples were then considered. As seen in Table 2, each 9.41 μg/mL sample produced an "H" value greater than the value of "3a." This indicates that at a concentration of 9.41 μg/mL mitragynine peaks are reproducibly discernable from the baseline noise, therefore qualifying the concentration of 9.41 μg/mL as the lower limit of detection for this experiment.

Reproducibility

The same standard was run a total of 10 times on different days at a variety of times. The 10 retention times were within 0.067 minutes of each other, with a standard deviation of 0.019591. The three characteristic peaks in the mass spectra for mitragynine were consistent across all 10 samples. Towards the end of the study, it was noted that the solution had turned a slightly yellow color, as opposed to the clear color when initially prepared; however, this does not seem to have affected the reproducibility of the mitragynine analysis. The dates, times, retention times, and primary mass spectra peaks can be seen in Table 3 below.

Table 3 Sample Analysis Date and Time, Mitragynine Retention Time, and Primary Mass Spectra Peaks of Mitragynine Used for Reproducibility Study

Date	Time	Retention Time (minutes)	Primary Mass Spectra Peaks
Monday, Dec. 15th	5:37 am	24.804	214, 397, 383
Monday, Dec. 15th	6:39 am	24.799	214, 397, 383
Monday, Dec. 15th	7:41 am	24.800	214, 397, 383
Sunday, Dec. 21st	2:30 pm	24.777	214, 397, 383
Sunday, Dec. 21st	6:50 pm	24.777	214, 397, 383
Sunday, Dec. 28th	10:18 pm	24.789	214, 397, 383
Monday, Dec. 29th	9:25 am	24.803	214, 397, 383
Tuesday, Dec. 30th	7:18 pm	24.803	214, 397, 383
Wednesday, Dec. 31st	9:14 am	24.818	214, 397, 383
Thursday, Jan. 1st	11:52 am	24.844	214, 397, 383
		STD = 0.019591	

Table 4 Sample Name, Company Name, and Company Location of Samples Purchased from Amazon.com

Sample Name	Company Selling Sample	Company Location
Euphoric Select	Advanced Nutrition	Lake Jackson, TX
Formula 303	Low Prices and More	Dublin, OH
Green Borneo	DirectSale USA	North Hollywood, CA
Green MD	MicrofineK	Albany, OR
Maeng Da	Vision-Quest Organics	Portland, OR
Pain-RX	Hi-Tech Pharmaceuticals	Lexington, KY
Red Borneo	DirectSale USA	North Hollywood, CA
Red Vein Super Grade Herb	Gold of Sunshine (GOSB)	Portland, OR
VivaZen	VitaBoost	Atlanta, GA
White Borneo	DirectSale USA	North Hollywood, CA
White Borneo	TreeKompany	Mount Sterling, KY

The names and locations of the companies that the samples purchased on Amazon.com can be seen in Table 4 above. The liquid samples consisted of the three VivaZen samples and the O.P.M.S. Liquid Kratom. VivaZen and O.P.M.S. Liquid Kratom samples are pictured in Figure 4 below. The powdered samples analyzed were Green Borneo, Green MD, Maeng Da, Red Borneo, Red Vein, and two White Borneos. Going from left to right, Red Vein Super Grade Herb, Maeng Da, Green Borneo, Red Borneo, and White Borneo can be seen in Figure 5 below. Samples such as green, white, and red borneo are different strains of *Mitragyna speciosa*, and are named based on the color of the veins in the leaves the sample is prepared from. Maeng Da is a genetically enhanced variant of Thai kratom. Suppliers claim that as a result of the genetic modification, Maeng Da is one of the most potent forms of kratom.[34] Finally, the package of Euphoric Select contained two capsules, which when opened, appeared to contain powdered plant material.

Figure 4 O.P.M.S. and VivaZen Samples

Figure 5 From Left to Right: Red Vein Super Grade Herb, Maeng Da, Green Borneo, Red Borneo, and White Borneo Powdered Samples

Mitragynine was detected in the following twelve samples: Euphoric Select, Green Borneo, Green MD, Maeng Da, Red Borneo, Red Vein Super Grade Herb, all three VivaZen samples, O.P.M.S. Liquid Kratom, and the two White Borneo samples. 7-hydroxymitragynine was not detected in any of the samples analyzed in this experiment.

The mass spectra of mitragynine in the experimental samples matched that of the mitragynine standard received from Cerilliant, as well as the mass spectrum present in the NIST05 library with 93% likelihood. A chromatogram of the red vein sample with the mass spectrum of mitragynine can be seen in Figure 6 below. The peak eluting at 24.944 minutes is identified as mitragynine because the ratio between the 397 ion and the 383 ion is greater than one. This is consistent with the method determined by Wang et al. to differentiate between mitragynine and speciociliatine, which co-eluted under the conditions of his method.

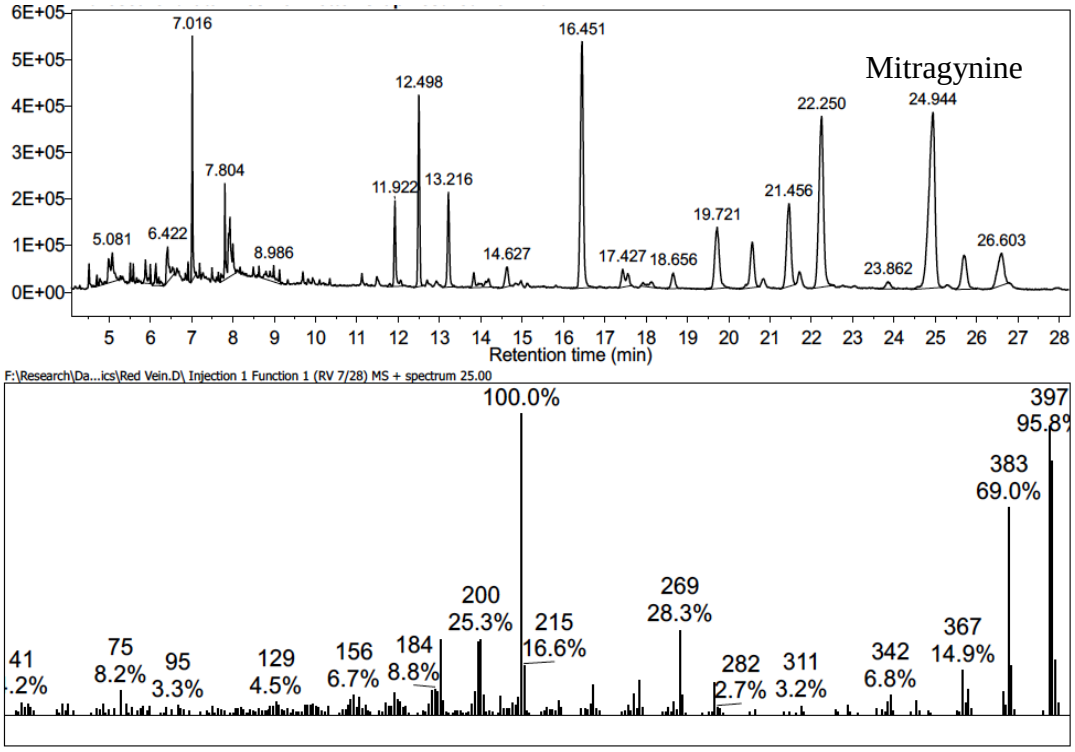

Figure 6 Chromatogram of Red Vein Super Grade Herb Sample (top) and Mass Spectrum of Mitragynine (bottom)

In addition to mitragynine, there were several additional peaks present in all sample extracts. An attempt was made via comparison to previous literature, as well as

library searches, to identify the peaks. The three-peak pattern containing mitragynine at 25.944, and peaks at 25.621, and 26.603 appeared in most of the chromatograms. The peak eluting after the mitragynine peak in at $t_{(r)}$ = 25.621 was identified to be paynantheine or 3-isopaynantheine by comparing the mass spectrum to the mass spectrum of paynantheine and 3-isopaynantheine published by Wang et al.[25] The last peak in the pattern, $t_{(r)}$ = 26.603 minutes, is tentatively as mitraciliatine via a NIST05 library search with a match quality of 95%. Mitraciliatine is an isomer of mitragynine. Other peaks in the spectrums were determined to be plant sterols such as stigmasterol and campesterol, as well as some fatty acids, by comparison to the NIST05 library.

The two samples in which mitragynine was not detected, Pain-RX and Formula 303, were both in pill form. These two samples did not list mitragynine or *Mitragyna speciosa* as an ingredient, but were purchased for analysis because they consistently appeared as hits on Amazon.com when kratom was used as the search term. The Formula 303 is listed as 'pain relieve natural muscle relaxant 90 tablets' for muscle spasm & leg cramps, tension, stress, anxiety, jitters, insomnia, back spasm TMJ, fibromyalgia, and menstrual cramps. It is purported to contain valerian root, passiflora and magnesium carbonate.[35] The Pain RX is advertised as containing plant extracts and user reports claim that it is 1/2 to 2/3 as effective as ibuprofen. Again, neither claimed to contain kratom or mitragynine, even though they were hits on a search for kratom.

It is interesting to note that during the time frame of this research there was a significant decline in results for a search for kratom on the Amazon.com web pages, although this trend reversed in the early months of 2015.

Analysis of VivaZen Samples

The VivaZen samples were of particular interest in this experiment because it is being marketed as a pain reliever. A study in 2007 noted that more than 30% of U.S. patients use herbal remedies, and a large majority of those remedies are used for conditions involving chronic pain.[5] This indicates a potential shift in the market of kratom from recreational powders and capsules to pain relievers. The initial VivaZen samples were purchased from Amazon.com and a local convenience store. Both of these samples contained mitragynine. On a subsequent visit to the same convenience store, the owner disclosed that several customers had complained about a lack of effects of the VivaZen as compared to earlier purchases. Upon inspection of the labels on the VivaZen bottle, it was observed that the sample from Amazon.com and the first sample from the convenience store listed *Mitragyna speciosa* as the first ingredient. The back label of the second bottle obtained from the convenience store listed *Mitragyna speciosa* leaf extract as the first ingredient. There were also several other ingredient changes. The first bottles included ingredients such as sacred lotus, skullcap, boswellia, and wild dagga. These ingredients were replaced yohimbe bark extract and valerian root extract in the second convenience store VivaZen sample. The proprietary blend did increase from 750 mg in the Amazon.com and first convenience store sample to 1.03 grams in the second convenience store sample. The second convenience store sample also had different warnings associated with it. The first two samples stated to not exceed two bottles in a twenty-four hour period, whereas the second convenience store sample stated to not exceed one bottle in a forty-eight hour period. The second convenience store sample also

included an additional warning against consuming the product for more than four consecutive weeks.

Even though there was differing information between the two sets of samples, the second convenience store sample did also contain mitragynine and the same repetitive three-peak pattern that occurred in all samples. Qualitative analysis indicates that the VivaZen sample received from Amazon.com contained about twice the concentration of mitragynine than the two samples from the convenience store. This could coincide with the complaints from customers that the convenience store owner was receiving about the VivaZen not being as effective as it used to be. Several reviews on Amazon.com from May 2014 to August 2014 have discussed the change in the formula of VivaZen. Customers noted a change in the amount of active ingredients during that timeframe and have attributed the change to the lessened effects of recent VivaZen.

When extracting the liquid samples, both the VivaZen and the O.P.M.S. Kratom, the acidic layer was analyzed in an effort to determine if compounds were being removed before the basic extraction step. It is interesting to note that mitragynine appeared in both the acidic and basic extract layers. An explanation for this might be that mitragynine is a neutral drug, similar to caffeine, and would therefore extract in either the acidic or basic layer depending on which pH adjustment was performed first.

CONCLUSIONS

The aims of this project were to create a GC-MS method to identify kratom that could be adopted in forensic drug laboratories, and to test the method on samples that might be commonly submitted to forensic laboratories. The method, as developed, will simultaneously detect mitragynine and 7-hydroxymitragynine, although 7-hydroxymitragynine is present in such low levels in the plant material that it would not be detected using sample sizes generally tested in forensic laboratories. This was not considered an issue as standard operating procedures only test for one substance for drug identification. For example, testing for Δ^9-tetrahydrocannabinol is sufficient for the identification of marijuana.

The developed GC-MS method is a successful method for the analysis of small kratom samples. The method successfully detected mitragynine, paynantheine, and/or 3-isopaynantheine. Since these alkaloids are specific to *Mitragyna speciosa*, identification of a sample as kratom can be achieved. Even though kratom is still currently legal in the United States, it is important for forensic chemists to be able to accurately analyze kratom samples in states where it has been controlled or in the event it were to become federally controlled.

Mitragynine was successfully detected and identified in all 12 samples that indicated mitragynine or *Mitragyna speciosa* as an ingredient. The two samples in which

mitragynine was not detected did not have mitragynine or *Mitragyna speciosa* listed as ingredients.

Several VivaZen samples were analyzed in this experiment for a couple of reasons. The first reason was to determine if the sample received from Amazon.com was the same as the sample received from a local convenience store. The second reason focused on customer complaints that VivaZen product not being as effective as it had been in the past. Upon closer inspection, it appeared that the proprietary blend had indeed been changed. The new sample was analyzed to determine if mitragynine had been removed, or if 7-hydroxymitragynine had been added. Mitragynine was still detected, though at a lower concentration than the initial sample, and there was no indication of the inclusion of 7-hydroxymitragynine.

Future Research

Furture research into the method for identifying kratom includes determing the limit of quantitation and investigation of a more appropriate internal standard. Dibucaine was selected because it is the internal standard used by the Alabama Department of Forensic Sciences, but it is not the best internal standard for researching methods for mitragynine and 7-hydroxymitragynine analysis. Deuterated mitragynine and 7-hydroxymitragynine standards are available and would be more useful for qualitative as well as quantitative analyses than dibucaine.[36,37] These standards would be separable from mitragynine and 7-hydroxymitragynine in the gas chromatography column, but would also react similarly to environmental influences and changes that might occur.

Decreasing the GC run time would also improve the method. Increasing the intial temperature and the ramp rate early in the run could potentially decrease the retention time and increase the quality of the mitragynine peak. It would also be interesting to analyze fresh plant leaves in small sample sizes to determine if extraction of the leaves would yield any detectable levels of 7-hydroxymitragynine, since previous research has successfully extracted them from large sample sizes of fresh leaves.

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