Quantitative Analysis of Mitragynine in Consumer Products Labeled as Kratom

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THESIS

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This thesis is dedicated to my wife, Kristin, and my children, Lucas and Leah, whose love and support provide the foundation for everything I do.

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LIST OF ABBREVIATIONS

7OH-MG	7-hydroxymitragynine
ALT	Alanine aminotransferase
CORY	Corynantheidine
DCM	Methylene chloride
FDA	Food and drug administration
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GDP	Gross domestic product
IS	Internal Standard
LC	Liquid Chromatography
LC-MS	Liquid Chromatography Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantitation
MeOH	Methanol
MG	Mitragynine
O.P.M.S	Optimized Plant Mediated Solutions
PAY	Paynantheine
SPG	Speciogynine
SPC	Speciociliatine
SWGDRUG	Scientific working group for the analysis of seized drugs

SUMMARY

Qualitative and quantitative analysis of commercially purchased products labeled "kratom" were completed using an Agilent 6890N gas chromatography instrument with an Agilent 5975 mass spectrometer. Mitragynine and 7-hydroxymitragynine, the two psychoactive components in kratom, were quantified as well as paynantheine, an alkaloid also present in kratom. 32 products were tested of which 22 were in powder form, 5 were capsules containing powder, and 5 were in a liquid form. All samples were extracted using 100% methanol with a 30 minute sonication period.

All 32 products contained mitragynine, 30 of which could be quantified. They ranged in concentration from 3.2 to 12.5 g/mg. 13 of the 32 products had detectable levels of 7-hydroxymitragynine, 7 of which could be quantified. They ranged in concentration from 2.02 to 2.03 mg/g. 30 of the 32 products had detectable levels of paynantheine, 23 of which could be quantified. They ranged in concentration from 2.3 to 6.7 mg/g.

Six of the products had a reported amount of mitragynine present written on the packaging. All 6 of these products were under the concentration that was reported on the packaging. The products with the highest labeled concentrations actually had lower calculated concentrations than some of their lower concentration labeled counterparts. More concerning was that the lowest labeled concentration product had a higher calculated concentration than almost all of the other products.

1. INTRODUCTION

1.1 **Mitragyna speciosa**

Mitragyna speciosa (korth.) is one of ten species of tropical evergreen trees native to Southeast Asia, more specifically Thailand, Malaysia, and Myanmar. The other 9 species are native to Southeast Asia as well as areas such as China, Bangladesh, India, Sri Lanka, and Africa (1). These species are usually broken down into an African or Asian species group with four and six of the plants belonging to each, respectively (1). The African species include M. inermis, M. ledermanii, M. rubrostipulata, and M. stipulosa, while the Asian species contain M. diversifolia, M. hirsute, M. parvifolia, M. rotundifolia, M. speciosa, and M. tubulosa (1). The Mitragyna genus is part of the Rubiaceae family, which contains about 13,000 different species throughout the world with the most notable being the coffee plants (1). However, M. speciosa is currently the only plant shown to have psychoactive effects when consumed due to the chemicals that are present.

All of the species of Mitragyna are very similar in structure and can often be confused with one another. In order to properly distinguish the species a proper examination of the flowers and fruits must be performed. For example, M. parvifolia and M. diversifolia are often mistaken for one another as well as M. stipulosa and M. ledermanii (1). Due to their very similar morphology, especially when it comes to the leaves, other species of Mitragyna have been substituted or sold as kratom (1). However, these ‘counterfeit’ leaves do not possess the correct chemicals to provide the same effects as that of M. speciosa (1, 2).

M. speciosa itself is a large arboreal tree that can reach up to 30 m in height (2) and can reach a spread of 15 m (3). It produces a spherical flower head that can contain up to 120 florets which, when pollenated, will create a small fruit-like capsule with small flat seeds (2). The

branches of the tree grow 10-12 leaves each, with the leaves being in pairs that form a right angle to each other (2). The color of the stems of the leaves can vary in color and are typically found in a shade from light green to red (2). The leaf itself is typically oval in shape coming to a single point at the terminal end. It grows in tropical areas where water is abundant (2).

1.2 **Ethnopharmacology**

The history of kratom use around the world is not well documented or known (4, 5). The kratom leaf use has a similar history to that of the coca leaf. The first recorded use of the leaf was in 1836 (2, 6, 7). The leaf was chewed by field workers in Malaya (currently Malaysia) and Thailand to prevent fatigue and increase their workload (5, 6, 7, 8). When chewed this way, it was noted that it produced a cocaine-like effect. Frequent users were reported to be darker skinned with a lower body weight than those who did not chew (5, 8).

There were multiple medical uses reported by the locals of these areas as well. The medical uses varied among the 10 species of mitragyna and utilized the many parts of the plant. The leaf, bark, and root were the most commonly used portions and treated fever and malaria. In addition the leaves, bark, and roots were ground into a paste and applied topically to treat skin ailments such as wounds, boils, eczema, blisters, and fungal infections (1, 4, 9). The natives also realized that boiling the leaves and consuming the water helped to reduce pain from numerous ailments (1, 4, 9). It is even noted that kratom could be used as an opium substitute (7).

It has been anecdotally suggested that the kratom leaf was first brought to the U.S. in the 1980’s and 1990’s by Hmong immigrants (4). However, the first written report of kratom use in the United States does not seem to have happened until 1999/2000 when it was first reported in the Entheogen Review (1, 10). Since then, interest in kratom has increased with a report of over

10,000 vendors currently selling kratom products legally in the U.S. (4). A Google Trends search indicates that interest in kratom was low in 2004 but has steadily gained interest with a more recent uptake in searches since the year 2016 (11). Today, it is estimated by the American Kratom Associations that 3 to 5 million Americans use kratom in some capacity (12, 13).

According to Paul Georgia, Ph.D., the kratom industry is responsible for about 4,640 jobs in the U.S. and brings in $342.1 million to the U.S. GDP annually when analyzing both direct and indirect contributions (13). This contributes $96 million in state and federal taxes each year (13). The kratom market also provides 4,640 jobs in the U.S, which provides $220 million in labor income (13).

There are many differences between the current use of kratom in South East Asia and that of the U.S. First, it is currently illegal in South East Asian countries and is the 2nd most abused illegal drug in Thailand (6). In the U.S. kratom is currently not federally illegal, but rather listed as a drug of concern. Another difference is in how kratom is acquired. In Asia, users typically have their own tree or personally know someone who has a tree growing and acquire it directly (5, 14, 15, 16, 17). In the U.S. most users buy kratom from the internet or a local head shop (5, 14, 15, 16, 17). Further, the fresh leaves in Asia are still typically chewed by the laborers, with only a few reporting that they brew a tea or the newer 4 x 100 cocktail for recreational purposes (5, 16). In the U.S., dry powder or pills are typically purchased with the former made into a tea and the later consumed directly (5, 14, 15, 16, 17). The use for kratom also differs between the countries. In the U.S. kratom is used to help treat chronic pain, or for recreational purposes while in South East Asia, kratom is used to help with manual labor as well as for medicinal purposes (5, 14, 15, 16, 17). Perhaps the most interesting difference is the reports of negative side effects between the countries. The majority of reports in South East Asia show only mild side effects,

such as constipation or skin pigmentation change with frequent use. In the U.S. kratom is being reported as causing seizures and the FDA is reporting deaths (5, 16, 18, 19, 20). This is very interesting considering that kratom has been used for a much longer period in South East Asia with no deaths being directly related to its use (5, 14, 15, 16, 17). This may be due to the media and drug laws in the U.S. versus South East Asia, or it may be due to how kratom is used.

1.3 **Typical Use**

The effects of kratom use have been reported to depend greatly upon the dose in which one consumes kratom. Numerous studies have indicated that a reported dose of 1-5 grams of kratom leaves, whether chewed or brewed into a tea, produce a stimulant-like effect comparable to that of the coca leaf. At a higher dose of 15 grams of leaves, kratom seems to have analgesic properties and appears to be opioid-like (4, 5, 12, 14, 16, 19, 21, 22, 23, 24, 25, 26). At these higher doses, users also experience constipation, addiction, and withdrawal like symptoms similar to that of morphine. However, the withdrawal experienced is much milder than that of opioids (5, 6, 7, 16).

In the U.S. Kratom is typically made into a tea and drank (5, 4, 15, 17, 24, 26, 27, 28). The process includes boiling kratom leaves or powder in water creating a tea. The brew time ranges and varies among reports from 5 minutes to 5 hours (5, 4, 15, 17, 24, 26, 27, 28). Users typically have a glass at a time about 3-5 times a day. The dose of mitragynine (MG) present in these drinks averages about 79 mg giving a total daily dose of 237-395 mg of MG. This can be drank warm or cold and is often mixed with sweeter beverages due to kratom's naturally bitter taste. Kratom leaves and powder can also be chewed or eaten, although this is a less popular option among users in the U.S. Kratom can also be purchased in a pill form or in a newer 'energy

shot' type of delivery. These types of kratom have relative strength comparisons listed on them, but these remain unverified.

There are reports of kratom leaves being smoked (4, 8). However, this appears to be extremely uncommon. Further, it has been shown that kratom has less bioavailability when used subcutaneously (2, 8). Thus, kratom is most often made by boiling the product and drinking the remaining liquid or by taking a pill form of the powder (5, 26).

Anecdotally, kratom has been purchased and used to help treat addiction to opioids (29). A 43 year old male, after being admitted to the hospital for a tonic-clonic seizure, admitted to spending about $15k a year on kratom for the purpose of ending a hydromorphone addiction (29). In fact, a study from John Hopkins University states that self-treatment like this may be beneficial and further research into the subject is needed (30). One such study was that done by Hemby et al. (31) which showed that kratom may be a treatment for opioid addiction. In this study, rats were first dosed with morphine until addicted and then administered MG and/or 7OH-MG. The rats decreased their use of morphine while taking the MG. Naloxone was shown to decrease the need for all of these drugs completely in dependent rats (31).

1.4 **Chemicals of Interest**

Since it was first studied in 1907, there have been 57 phytochemicals discovered to be contained in M. speciosa of which 37 are alkaloids (1, 32, 33, 34). Of all these chemicals, MG and 7-hydroxymitragynine (7OH-MG) have been identified as the two chemicals that have the most psychoactive properties when consumed (1, 9, 12, 14, 21, 27, 33, 35, 36, 37, 38, 39). Other chemicals have also been identified and suspected of having psychoactive properties such as paynantheine, mitraphylline, speciociliatine, and speciogynine (1, 9, 22, 33, 36). However,

research is limited into the role these chemicals play both alone and together when consuming kratom. Some studies have suggested that these chemicals have very low potency when compared to MG and 7OH-MG or have no effect at all. For example, corynantheidine shows no opioid like activity and actually inhibits the effects of morphine (32). Speciociliatine did show opioid like activity, but at a 13x lower potency than MG (32).

Concentrations of these chemicals vary greatly depending on where and how the plant is grown (1, 4, 40, 41). When looking at the total amount of alkaloids present in kratom, MG – which is the most abundant – ranges from 66% to 12% of the crude extract depending on if it is grown in the Thai or Malaysian region alone (1, 32, 33, 34, 41). 7OH-MG, the second most psychoactive component, accounts for only about 2% of the total alkaloids present. The remaining 32% is split among the other 35 alkaloids (1, 32, 33, 34, 41).

MG and 7OH-MG are the most heavily researched chemicals when it comes to M. speciosa. These two chemicals are also currently only known to be present in M. speciosa naturally and have not been found in any of the other 9 species of Mitragyna (1, 33, 37, 43). Both MG and 7OH-MG can be synthesized in a laboratory setting and have been described by Cook, Ma, Takayama, and Kerschgens (44, 45, 46, 47, 48). Further, it has been shown that MG exhibits auto-oxidation to become 7OH-MG and may do this via metabolism within the plant itself (42). It is suspected that MG is metabolized into 7OH-MG in the body during a first pass liver metabolism and may explain the potency of ingesting kratom (4, 14).

As for the chemicals themselves, MG is a white powdery substance with the chemical formula $C_{23}H_{30}N_2O_4$. Its IUPAC name is methyl (E)-2-[(2S,3S,12bS)-3-ethyl-8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl]-3-methoxyprop-2-enoate. Its molecular weight is 398.503 g/mol (49, 50).

7OH-MG is also a white powdery substance that has an extra hydroxy group attached to the 7th carbon on the ring structure of MG (33). Its chemical formula is $C_{23}H_{30}N_2O_5$. Its IUPAC name is methyl (E)-2-[(2S,3S,7aS,12bS)-3-ethyl-7a-hydroxy-8-methoxy-2,3,4,6,7,12b-hexahydro-1H-indolo[2,3-a]quinolizin-2-yl]-3-methoxyprop-2-enoate. Its molecular weight is 414.502g/mol (51).

1.5 **Toxicity**

The toxicity of these chemicals and of kratom as a whole is still unknown. The FDA has reported as many as 44 deaths since 2005 in the U.S. from the use of kratom. However, investigation into these deaths reveals that there is usually another drug present, or that kratom was present in someone's system who was murdered or committed suicide (4, 19, 20, 52, 53, 54, 55, 56, 57). Currently, there have been no deaths that were solely caused by the consumption of kratom. Of the deaths that were reported, blood concentration levels of MG have been 600 μg/L, 230 - 1006 μg/L, 10 μg/L, 790 μg/L, and 230 μg/L (54, 57, 56). One report indicated that a blood concentration level of 0.167 mg/L caused a seizure (56). However, to date there has been no scientific study showing the toxic level of MG or any other chemical consumed with kratom use (20).

Calls to the poison control centers for kratom use have been steadily increasing in the U.S. In 2016 there were 97 reported calls to the poison control centers for kratom. So far in 2018 there have been a reported 635 calls (58). However, only 3 of these calls have been due to kratom alone. The majority of calls are due to kratom being taken with other drugs or alcohol (58).

There have been a range of reports in terms of the analgesic properties of MG and 7OH-MG. A study conducted on guinea pigs by Stolt et al. (22) showed that MG had a weak potency when administered alone, but 7OH-MG showed 30x more potency than MG when administered. Further, 7OH-MG showed a 17x increase in analgesic activity when compared to morphine (22). Similarly, a study done by Michael White showed that 7OH-MG was 46x more potent than MG and 13x stronger than morphine (12). Lydecker et al. (42) compared the potency of MG to morphine saying it was 1/3 as potent as morphine, but 3x more potent than codeine. They also stated that 7OH-MG was 17x as potent as morphine (42). However, Kruegel and Grundmann (9) stated that MG was about as potent as codeine and that 7OH-MG was 10-20x more potent than morphine (9). Hemby et al. related the potency to the different opioid receptors that MG and 7OH-MG target. They discovered that 7OH-MG has a 5x greater affinity for the μ-opioid receptor when compared to MG and a 40x greater potency than MG. Further, when comparing 7OH-MG to morphine, they stated that it had a 10x greater potency (31). Although all of these studies and reports have slightly different numbers, it appears that 7OH-MG is the much more potent of the two chemicals and both exhibit analgesic properties.

When taken orally, users can expect to feel the effects of kratom at about the 10-20 minute mark with the effects peaking at about 30-60 minutes (4, 14). MG and 7OH-MG have been shown to have half-lives of about 3.5 hours and 2.5 hours, respectively, and both are removed from the body via urine (4, 14).

Toxicity studies have been completed using rodents. In a study by Harizal et al. (35), kratom extract concentrations of 100 mg/kg, 500 mg/kg, and 1000 mg/kg were administered once to rodents orally and they were observed over a 14 day period. None of the rodents died during this study. However, it was observed that the 1000 mg/kg dosed rodents had rapid

breathing and slowed movements lasting about 30 minutes after consumption. All rodents showed increased blood pressure readings, as well as increased ALT (Alanine aminotransferase), albumin, and triglyceride levels indicating liver injury. This was confirmed by histology examination of the organs which revealed Kupffer cells and karyomegaly. However, the lower doses were not as significant as the 1000 mg/kg dose. Cholesterol, urea, and creatinine levels were also increased, but not to the point of kidney damage. In conclusion, this study found that the highest dose leads to acute severe liver hepatotoxicity and mild nephrotoxicity after a single dose administration of kratom extract. The control group of rodents were administered morphine. These rodents showed much more severe changes than any of the MG administered rodents.

In a rodent study by Azizi et al. (14, 28) rodents that were given a total alkaloid extract of kratom – a purified version of the chemicals present – died at the 200 mg/kg dose. However, this was not discussed in great detail in the study making it unknown what all directly contributed to the rodent's death. Currently, there is no known lethal dose of MG or 7OH-MG in humans.

Kratom has been found to cross to the fetus if consumed while pregnant (52). A mother admitted to kratom use during pregnancy and after birth the infant presented to the hospital with signs of opioid withdrawal (52). These included irritability, jitteriness, muscle hypertonicity, and breathing difficulties. The mother reported using kratom only "occasionally" to help her relax (52). The baby was treated with methadone and IV glucose successfully.

Kratom may have an increased effect when mixed with other drugs. As previously stated, the FDA has reported as many as 44 deaths since 2005 in the U.S. from the use of kratom. The majority of these are linked to a mix of drug use (4, 19, 20, 52, 53, 54, 55, 56, 57). Two of the most commons ways kratom is mixed with other drugs are 'Krypton' and the 4 x 100 kratom cocktail, named so because of the 4 ingredients that are traditionally used. Krypton is a mixture

of kratom with O-desmethyltramadol, which is an opioid agonist itself (1, 4, 37, 39, 59, 60). The 4 x 100 cocktail is a mixture of kratom, ice, a cola product, and a cough syrup product that contains codeine or dextromethorphan (1, 5, 17, 59, 61).

1.6 **Other Chemicals Present**

As previously stated, there are about 57 phytochemicals present in M. speciosa of which 37 are alkaloids (1, 32, 33, 34). The two major chemicals are MG and 7OH-MG which make up about 66% and 2% of the total alkaloid content, respectively. Of the other major alkaloids present, paynantheine, speciogynine, and speciociliatine make up about 9%, 7%, and 1% of the total alkaloid content. The alkaloids mitraciliatine, corynantheidine, and 9-corynantheidine are also frequently referred to although they both make up less than 1% of the alkaloid content (1, 9, 32, 60, 62). Most of the alkaloids present are diastereomers of MG. These 8 alkaloids are displayed in figure 1.

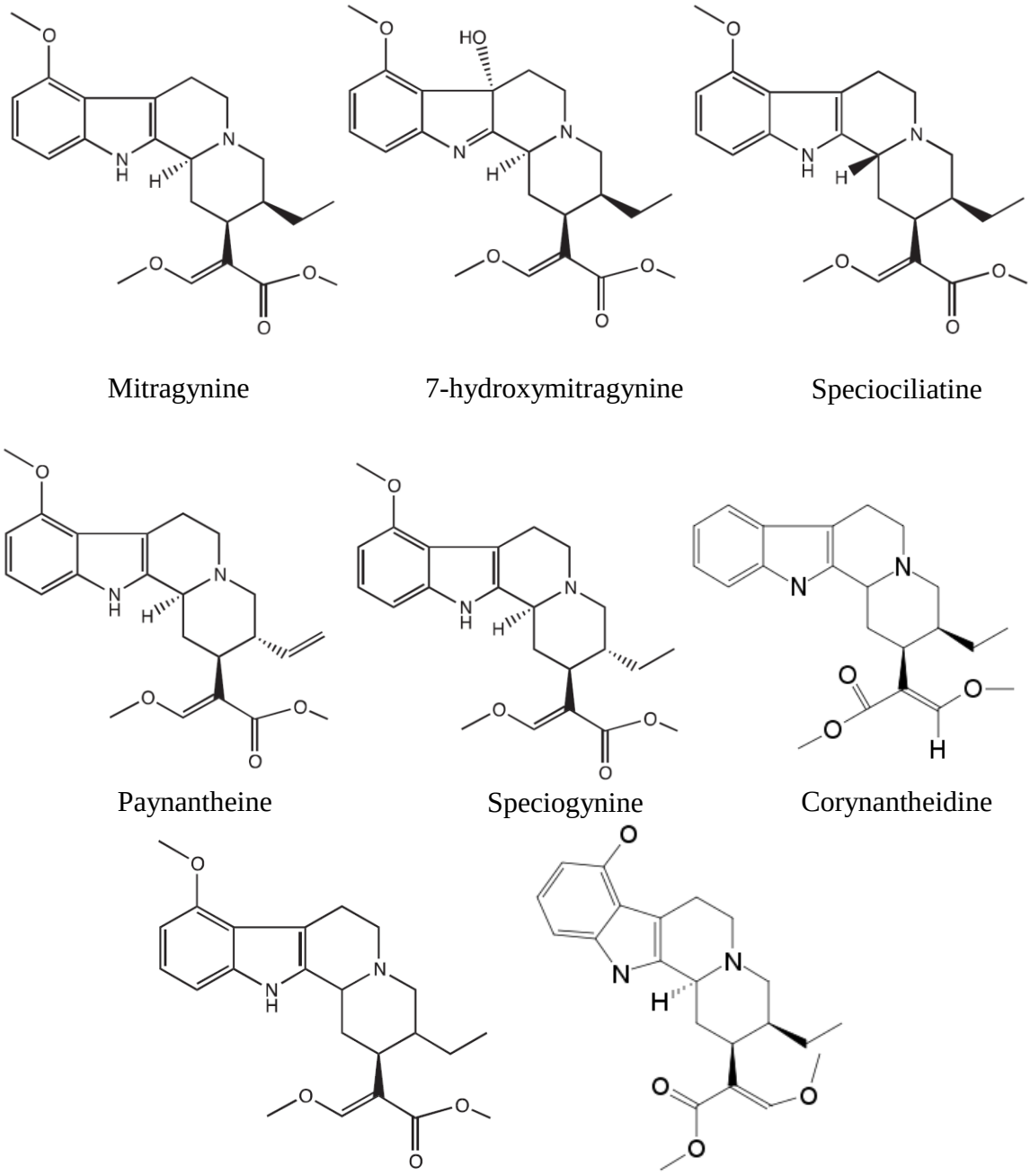

Figure 1. Chemical structures of the most commonly referred to alkaloids found in M. Speciosa.

Research on these different alkaloids is very limited with most of the research being concentrated on MG and 7OH-MG due to their abundance in kratom as well as their proven psychoactive effects. However, some research has been done on other alkaloids. One such alkaloid is speciociliatine which is a diastereomer of MG at the C3 position (60). Speciociliatine was shown to have an opioid like effect mimicking that of MG, but its potency was 13x lower than that of MG (32). Speciogynine and paynantheine also showed opioid agonist activity, but with lower potency than that of speciociliatine.

Corynantheidine did not show opioid activity, but in fact was antagonistic when used with morphine (32). 9-Hydroxycorynantheidine on the other hand showed opioid agonistic activity, but with lower potency than that of MG. This suggests that the C9 functional group may be key to the activity of these MG related compounds as a whole (32, 63).

1.7 **Legality**

Kratom is currently under legal scrutiny throughout the world. It was first banned in 1943 from the plant's home country of Thailand. The government passed the "Kratom Act 2486" which made the planting of the tree illegal (3). It was further legislated against by the Thai government with the passing of the "Narcotics Act B.E. 2522" which placed it into Category V of a five category system. This system is similar to the United States Drug Enforcement Agency scheduling in that category V is the lowest category containing the least harmful substances (64). Kratom is still a popular drug in Thailand with reports of people creating the 4 x 100 cocktails (2, 3, 61). Recently, the Thai government has considered reversing their ban due to the promising taxes that could be collected, but it has not done so to date (65).

Malaysia has banned the use of kratom leaves through section 30(3) of their Poisons Act of 1952 (66) with a punishment of up to 4 years in jail and a fine of about $3,150 U.S. dollars.

There was a push to make kratom use even more punishable through Malaysia's Dangerous Drugs Act, but this act failed in 2015 (67).

More recently, the European countries of Denmark, Latvia, Lithuania, Poland, Romania, and Sweden all have some type of ban on kratom use (68). The U.K. has banned kratom under its Psychoactive Substances Act of 2016 (69). Canada also has banned the sale of kratom for human consumption, but the law allows for the sale of kratom as long as it is not marketed for human use (70).

Australia and New Zealand similarly have banned kratom. New Zealand did so under its Medicines Regulations of 1984, which was revised in 2017 (71). Australia placed kratom as a controlled narcotic in 2005 (72).

The Association of South East Asian Nations (ASEAN) has also banned the trade and sale of kratom as a traditional medicine or health supplement (73). This means that it cannot be sold or traded among the nations participating in ASEAN, which includes Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand, and Vietnam.

The World Anti-Doping Agency, responsible for the monitoring and testing for illegal drugs in professional sports, has placed kratom on its monitoring list as of 2014 (38). This is because it may give athletes the ability to perform for longer due to its effects.

The United States of America has gone back and forth on the decision to either ban or legalize kratom with lobbyists and research supporting both sides coming forward. Currently, kratom is not banned federally in the United States and it is not on the controlled substance list. However, Alabama, Arkansas, Indiana, Rhode Island, Vermont, Washington D.C., and Wisconsin, as well as some specific cities in California, Colorado, Florida, and Illinois have banned kratom sale and use (26, 74). The FDA has issued a public health advisory over their

concerns on the “deadly risks” associated with kratom. They point to a lack of research and how it is currently being used to treat medical symptoms without review of a licensed physician (19). Furthermore, the FDA has attributed about 44 deaths to kratom use (18, 19). However, these deaths have been refuted, as previously stated (4, 20). Other sources have stated that the FDA is being too cautious and that kratom is not as harmful as they are saying (4, 18, 26). These same sources also argue that more research is necessary and that kratom may lead to new and better alternatives for both opioids as well as opioid withdrawal treatment (4, 18, 26, 32).

The Drug Enforcement Agency was going to emergency schedule kratom under schedule I of the Controlled Substances Act (26, 75). However, groups such as the American Kratom Association were able to lobby legislators and the DEA instead placed kratom into its “drugs of concern” pending further study (25, 26). This allows kratom to still be freely sold from the internet as well as in established businesses around the U.S.

1.8 **Current Research and Methods**

Research on the detection and quantification of kratom, more specifically MG and 7OH-MG, from raw materials as well as prepared products from the consumer market are scarce. Most of the current studies are examining whether or not MG and 7OH-MG are actually present in the samples considering the current U.S. consumer market is not regulated. There are only a few studies that quantify the amount of MG present, but the amount of products tested in these studies is limited. Some of these studies test samples of pure leaf from the actual kratom tree itself rather than purchasable products.

Currently, there are both GC-MS and LC-MS methods available for the detection and quantification of both MG and 7OH-MG. Some of the studies have brought up that GC-MS may

not be the best for quantification as speciociliatine could not be separated from MG (76, 77, 78). However, GC-MS is the current SWGDRUG method for the detection of MG in both drug chemistry and toxicology sections of forensic laboratories (63, 78). In addition, it was noted that speciociliantine could be differentiated from MG based on the ratio of the 397 and 383 ions. A study done by Fuenffinger et al. examined the use of ion-mobility spectrometry for the detection of MG as well as other alkaloids for the rapid screening of suspected kratom samples (41). They created a method that was able to determine if MG was present in kratom extracts in the presence of speciociliatine.

The majority of the quantitative studies for the use of kratom used LC-MS for quantification of the alkaloids present (37, 42, 76, 77, 79). These studies examined multiple types of kratom including raw kratom obtained from the tree itself, pure alkaloids that were purchased, and samples purchased from the commercial market. They were able to quantify MG and 7OH-MG and also identify the other alkaloids typically present. One study by Oliveira et al. was able to use GC-MS to quantify MG and distinguish between the other alkaloids present, but needed to use a slightly different extraction method for proper resolution (63).

Regardless of the type of study being done, they all had a similar type of extraction method. Methanol was the solvent of choice with some type of shaking, sonicating, or vortexing of the sample before allowing it to sit overnight (28, 33, 41, 42, 62, 76, 77, 78, 79, 80, 81). There were some slight variations in the amount of shaking, sonication, and vortexing times as well as the use of 80% or 100% methanol. However, Mudge and Brown used a wide range of methanol concentrations and used both water and 0.5M acetic acid to dilute the methanol (77). They determined that 70-80% methanol in water was best for the extraction of the kratom alkaloids and other studies seemed to have used this concentration (77, 78, 79, 81).

The studies that did not use methanol to extract the kratom alkaloids from the kratom products did not use a uniform solvent. Oliveira et al. used a 1:2 chloroform to methanol solution which allowed them to achieve better resolution and use GC-MS to quantify MG (63). Kowalczuk et al. used ethanol which also allowed them to do a microscopic examination of the ground kratom products themselves (80). Griffin et al. used chloroform for their solid extraction and then used an acid/base extraction method for the liquid kratom samples (78). This involved 0.1M HCl to bring the liquid samples to a pH of 1 followed by adding chloroform to the solution. The aqueous portion was then removed and brought to a pH of 10 with 4M NaOH. This was again followed by adding chloroform. The chloroform layer this time was extracted and tested. However, 7OH-MG was not detected during the GC-MS analysis during this method (78).

1.9 **Chromatographic Techniques**

The root of chromatography can be traced back to 1903 when botanist Mikhail Tswett used column chromatography to separate the various pigments present in plants (82). In fact, the Greek roots of the word chromatography relate directly to his work as *chroma* means “color” and *graphein* means “to write” (83). His method was so well written and defined that is was used unmodified for over 40 years (82). However, today’s modern chromatographic techniques can be attributed to the 1950’s when thin layer chromatography and gas chromatography were first introduced followed shortly thereafter by liquid chromatography (82).

Chromatography has always been a method for separating samples into their individual components. This is accomplished differently by the various techniques that are encompassed by chromatography, but in general they all include a stationary phase and a mobile phase. Samples are pushed through the stationary phase by the mobile phase and, based upon the interactions of

the various components in the sample, they move through the stationary phase at different rates resulting in their separation (82). These different techniques not only allow for the separation and detection of various components in a mixture, but can also be used with various detectors to give accurate quantitative analysis (82). For the purposes of this study, gas chromatography (GC) and liquid chromatography (LC) are described herein.

1.9.1 **Gas Chromatography**

Gas chromatography is named so because the mobile phase used in this technique is a gas. GC works by injecting a liquid sample into the instrument where it is heated into its gaseous state. This is then pushed by an inert gas into the column. The sample is separated while moving through the column as its different components interact with the stationary phase present at different rates. This causes them to exit the column and enter into the detector at different times. This is what allows for the detection of multiple compounds in a single sample.

A typically GC instrument consists of the same parts which includes an injection port, flow regulator, column, and detector (82). The injector port is usually split, or split less, but can also be an on-column injector. A split injector is the oldest of the injector types (82). About 1 microliter of sample is injected into the injector where it is immediately vaporized and then only about 0.1%-10% of the sample is pushed into the column while the rest is purged out of the system (82). A split less injector requires a larger amount of sample – about 1-5 microliters – that is diluted prior to being injected (82). The sample is still vaporized immediately but is pushed onto the column at a slower rate with the help of the flow regulator. This allows for about 20-50x the amount of sample being analyzed which increases sensitivity of the instrument (82). An on-

column injector is when sample is introduced directly into the column without the use of an injection port.

The injection port, column, and detector are what are heated during the process. The GC can be programmed to increase temperature during a run, which increases the speed at which analytes move out of the column and onto the detector (82). When using a split less injection, this temperature variation is vital for the separation of components from their diluting liquid. Temperature and column length are the most important factors when using GC as they can increase or decrease retention times and separation or analytes (82).

The typical mobile phase used in GC to push samples through the instrument includes nitrogen, helium, and hydrogen, with helium being the most common (82). These gases are pure and are chosen because they are inert, which is mandatory since their only job is to move analyte through the column. The flow rate of these gases is set by the flow regulator.

Gas chromatography is the most widely used chromatographic technique in the world today as well as the most common instrument used in forensic laboratories (82). It is fast, relatively inexpensive, can be automated, uses a small amount of sample, and allows for quantification of compounds (82). However, it is not well adapted for thermally labile compounds and can lack the sensitivity of LC.

1.9.2 **Liquid Chromatography**

Liquid chromatography is named so because the mobile phase used in this technique is a liquid. Liquid chromatography can be broken down into planar, which would include thin layer chromatography, and column. Column liquid chromatography is of importance to this study, more specifically, high pressure LC (HPLC) and is what is described.

Liquid chromatography works by introducing the sample to the instrument. This is then mixed and pushed into the column with the mobile phase, which is a liquid. The sample will be separated into its components based on the interactions with the mobile phase and the stationary phase. This causes them to exit the column and enter into the detector at different times. This is what allows for the detection of multiple compounds in a single sample.

It is important to note that the mobile phase in LC is not inert like the mobile phase present in GC (82). Because of this, there are two type of liquid chromatography which are normal phase and reversed-phased. Normal phase LC uses a polar stationary phase and a nonpolar mobile phase (82). Reversed-phase LC uses a nonpolar stationary phase, and a polar mobile phase (82). The most commonly used is the reversed-phased LC, which is ironic since that would make it the 'normal' version of LC.

A typical LC instrument is made up of a solvent reservoir, injector, pump, column, degasser, and detector (82). The solvent reservoir simple houses the solvents that are being used in the isocratic or gradient method you are using. The pump is the most important part of the instrument as it controls the pressure and flow of the mobile phase (82). Most pumps will cause a slight increase in pressure as they run due to their design which can produce noise in the results. However, most systems have mechanical and electronic dampeners to help remove this (82). The injector is where the sample is mixed with the mobile phase. The injector cycles during operation and will preload the column with mobile phase that is being used before switching to inject sample. This will happen numerous times during a run to ensure proper mixture of sample and mobile phase (82). The degasser is required to help remove oxygen from solvents and sample as oxygen can interfere greatly with the results on the detector (82).

Unlike GC, LC does not use a temperature range to assist with separation of components in a sample. However, LC can use an isocratic or gradient elution method. Isocratic elution use the same mobile phase throughout the run while gradient elutions would have the mobile phase from a ‘strong’ to ‘weak’ solvent/polarity, or vice-versa, during analysis (82). In a similar manner as the temperature range in GC, a gradient can be helpful in separating components from each other as well as making them elute out of the stationary phase (82). Gradients employ multiple mobile phases to change the strength or polarity while isocratic methods will use just one mobile phase. Determination of the proper mobile phase is often the most difficult part of the LC procedure as it has the greatest effect on analysis.

Liquid chromatography has the advantage of being able to analyze volatile compounds as well as more sensitive for quantitation. However, it is usually time consuming, has a more complex procedure, can have toxic or harmful chemicals, and is often more expensive than GC instrumentation (82).

1.10 **Mass Spectrometry**

Mass spectrometry (MS) is the most commonly used method for the qualitative and quantitative analysis (82). It is often combined with a chromatographic technique such as GC and LC which were discussed previously. The combination of these type of methods allows for both the separation of mixture components as well as the analysis of the components themselves. This allows for the qualitative and quantitative analysis of mixtures. These techniques are often hyphenated as Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LCMS).

When attached to a GC or LC, a mass spectrometer works by introducing the separated analytes to an ion source. The ion source then impacts the individual molecules present and breaks the bonds apart creating fragments. There fragments and their ion charge is detected and reported in a mass to charge ratio (m/z). This produces a mass spectrum for a specific analyte that is reproducible allowing for quantitative and qualitative analysis.

A typical mass spectrometer consists of an ion source, an analyzer, and a detector (82). The ion source of the instrument depends on whether it is being used with GC or LC. For GC-MS, the ion source is either an electron impact or chemical ionization (82). Electron impact is the oldest and most common used and uses electrons to bombard the analyte and cause fragmentation (82). Chemical ionization requires a reagent gas that becomes ionized and causes gas molecules to further bump into each other to create proton and hydride ions (82). These then interact with the analyte.

For LC-MS, the mobile phase needs to be removed from the sample prior to it being bombarded with electrons. This is accomplished by either a thermospray ionization (TSI), electrospray ionization (ESI), or atmospheric pressure ionization (API) type (82). All three of these ionization methods are similar in that they cause the liquid from the LC to create a fine mist or vapor which can then be bombarded with electrons to fragment the analyte.

The analyzer is used to separate the ions that are created by the ion source and come in a few variations. These types include the quadrupole, ion trap, and time of flight (82). Quadrupoles and ion traps work similarly in that they only allow the ion with the correct mass to charge ratio to pass through to the detector. However, the quadrupole uses 4 sections while the ion trap uses one (monopole). Time of flight analyzers separate these ions based on the amount of time it takes for the ion to go across a fixed distance (82). This is affected by the size of the ion.

1.11 **Purpose of this Study**

Many commercially available products containing kratom are being sold in the United States. These come in powders, pills, and liquids and vary greatly in name, branding, and claimed alkaloid content. These products are not regulated by the FDA and only some of the packages describe the alkaloid content and effects. This study will attempt to examine these products and quantify the alkaloids present – mainly the active alkaloids of MG and 7OH-MG.

This will be done by creating a GC-MS method that can accurately and rapidly detect the alkaloids of interest while providing proper resolution to allow quantification; creating an extraction procedure for both liquid and solid samples that will allow for their analysis on GC-MS; and comparing the resulting alkaloid content between samples to determine if there is any significant difference.

Based upon the anecdotal evidence and current research, commercially available kratom products will contain the active alkaloids of MG and 7OH-MG, but will have very little significant difference between the products despite what the packages claim.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Commercially purchased kratom products

Products labeled as containing kratom were purchased from 5 separate Chicago area smoke shops. Table I contains the store the products were purchased from, the brand, how the product was labeled, what the product form was such as liquid or powder, and the amount contained in the package. All descriptions are recorded from the packaging of the products purchased. In total, 32 samples were purchased of which 22 were in powder form, 5 were powder in capsules, and 5 were liquid samples. Figure 2 is a representative picture of all the purchased products.

TABLE I

DESCRIPTION OF PRODUCTS PURCHASED

Store	Brand	Labeling	Type	Quantity
Chicago Vapor Zone 1410 N Milwaukee Ave, Chicago, IL 60622	Kratom One	Tri Force	Powder	1 oz.
	Kratom One	Red Horn	Powder	1 oz.
	Kratom One	Bali	Powder	1 oz.
Smoke Shop XXX, Inc. 1920 W North Ave, Chicago, IL 60622	O.P.M.S.	Liquid Kratom	Liquid	8 mL
	Tropical Kratom	Green Strain	Powder in capsules	7 capsules
	Tropical Kratom	White Strain	Powder in capsules	7 capsules
Diffused Galleria 1448 N Milwaukee Ave, Chicago, IL 60622	O.P.M.S. Silver	Malay Special Reserve	Powder	28.35 grams
	No brand	Red Borneo	Powder	28 grams
	No Brand	Green Malay	Powder	28 grams
	No Brand	Bali	Powder in capsules	30 capsules (15g powder total)
Mr. Nice Guy 2048 N Damen Ave, Chicago, IL 60647	Choice Kratom	Maeng Da	Powder in capsules	6 capsules (0.8g/capsule)
	Bintang	Speciosa Shot	Liquid	1.93 fl oz.
	Liquid K	N/A	Liquid	2 fl oz.
	Raw Kratom	Private Reserve Mitragyna Speciosa Extract	Liquid	5 mL
	Zen	Liquid Kratom	Liquid	8 mL
	Kratom Kaps	Indo	Powder in capsules	20 grams
	Mr. Nice Guy	Red Sumatra	Powder	10 grams
	Mr. Nice Guy	Red Gold	Powder	10 grams
	Mr. Nice Guy	Red Bali	Powder	10 grams
	Mr. Nice Guy	Red Borneo	Powder	10 grams
	Mr. Nice Guy	Green Bali	Powder	10 grams
	Mr. Nice Guy	Green Sumatra	Powder	10 grams
	Mr. Nice Guy	Green Vietnam	Powder	10 grams
	Mr. Nice Guy	White Borneo	Powder	10 grams
	Mr. Nice Guy	White Bali	Powder	10 grams
	Mr. Nice Guy	White Gold	Powder	10 grams

TABLE I (Cont.)

DESCRIPTION OF PRODUCTS PURCHASED

Store	Brand	Labeling	Type	Quantity
Tobacco Hut 543 St. Charles Road, Villa Park, IL 60181	Remarkable Herbs	Indo	Powder	1 oz.
	Remarkable Herbs	Bali	Powder	1 oz.
	Remarkable Herbs	Thai	Powder	1 oz.
	Remarkable Herbs	Maeng Da	Powder	1 oz.
	Remarkable Herbs	Malaysian	Powder	1 oz.
	Remarkable Herbs	Vietnam	Powder	1 oz.

Figure 2. A) Image of the range of packaging on powder, capsules and liquid products purchased. B) Image of the appearance of capsules and the range of colors for the powders.

2.1.2 **Reagents**

HPLC grade methanol (MeOH) was obtained from Fisher Scientific (Hampton, NH); Mitragynine (MG), 7-hydroxymitragynine (7OH-MG), and Paynantheine (PAY) standards were obtained from Cayman Chemical (Ann Arbor, MI).

2.1.3 **Instrumentation and Supplies**

Agilent Technologies 6890N Gas Chromatograph. Agilent Technologies 5975 Inert XL Mass Selective Detector. Agilent Technologies 7683 Auto Sampler and Injector. American Scientific Products Vortex Model S8223-1. Fisher Scientific Ultrasonicator Model FS30. Agilent Technologies MassHunter Quantitative Analysis Software, Version B.07.01. Fisherbrand P8 qualitative filter paper (filter paper). 10 mL volumetric flasks (numerous)

2.2 **Preliminary Methods**

2.2.1 **Powder Extraction Method**

The current literature varied in extraction methods for kratom samples (28, 33, 41, 42, 62, 63, 76, 77, 78, 79, 80, 81). In order to ensure complete extraction of MG and 7OH-MG, different techniques were examined. A sonication with overnight incubation, a sonication with no incubation period, and serial extraction method were the techniques chosen due to their popularity among the literature. All of the techniques used 80% MeOH in water. They also were conducted on a single source of kratom; the remarkable herbs Vietnam Kratom powder. This was to ensure that variation in content was limited. Approximately 0.1 g of this powder in 10 mL of 80% MeOH was used in all samples. These samples were all prepared in clean 10 mL volumetric flasks.

For the sonication with overnight incubation method three samples were prepared. These samples were each vortexed for 1 minute and then sonicated for 30 minutes. The samples were then allowed to sit at room temperature for a 24 hour period. At the completion of this incubation the samples were individually gravity filtered using filter paper into new 10 mL volumetric flasks. These samples were labeled as 24H1-1X, 24H2-1X, and 24H3-1X.

For the sonication with no overnight incubation method three samples were prepared. These samples were each vortexed for 1 minute and then sonicated for 30 minutes. The samples were then immediately gravity filtered using filter paper into new 10 mL volumetric flasks. These samples were labeled as SON1-1X, SON2-1X, and SON3-1X.

To test the serial extraction method, as well as if the 24 hour incubation or just sonication method was needed on a serial extraction, all six of the previous samples were used. The filter paper from the previous samples was allowed to air dry and the powder was then scraped off into new 10 mL volumetric flasks. These flasks were then filled with 10 mL of the 80% MeOH. All six of these samples were vortexed for a minute each and then sonicated for 30 minutes. At this point the three samples from the 24 hour incubation, now labeled 24H1-2X, 24H2-2X, and 24H3-2X, were allowed to incubate at room temperature for another 24 hours. The three samples from the sonication only method, now labeled SON1-2X, SON 2-2X, and SON3-2X, were gravity filtered with new filter paper into clean 10 mL volumetric flasks. At the completion of the 24 hour incubation, the 24 hour samples were filtered with new filter paper into new 10 mL flasks. This entire process resulted in a total of 12 samples.

All 12 of these samples were analyzed using GC-MS. It was found that the samples labeled 24H1-1X, 24H2-1X, 24H3-1X, SON1-1X, SON2-1X, and SON3-1X all had detectable levels of MG and 7OH-MG present. However, in the samples labeled 24H1-2X, 24H2-2X,

24H3-2X, SON1-2X, SON2-2X, and SON3-2X no MG or 7OH-MG was detected. This indicated that serial extractions were not required in order to adequately extract the compounds of interest as there were no detectable levels of these compounds.

In order to better compare the sonication only and the 24 hour incubation methods, new samples were created. Three different powders were used this time from Remarkable Herbs Vietnam Kratom, Mr. Nice Guy Red Borneo, and O.P.M.S. Silver Malay Special. Three samples for each of the 24 hour extraction method and sonication method were created for each sample like before. This resulted in 18 total samples labeled as RH24H1, RH24H2, RH24H3, RHSON1, RHSON2, RHSON3, NG24H1, NG24H2, NG24H3, NGSON1, NGSON2, NGSON3, OP24H1, OP24H2, OP24H3, OPSON1, OPSON2, and OPSON3. Once filtered, these samples were analyzed via GC-MS. The average peak area of the MG peak was examined to see the differences between the 24 hour incubation and the sonication only samples of each brand. The average peak areas were in more than a 98% agreement indicating that either method would properly extract the compounds of interest. To save time, the sonication only method was chosen and used for the remainder of the study.

The results from these preliminary analysis allowed for the powder extraction method that was used for the remainder of the study. This method uses 0.1 g of kratom powder with 10 mL of 80% methanol in water in a volumetric flask. This is then vortexed for one minute followed by a 30 minute sonication period. At the end of sonication, the sample is gravity filtered using filter paper into new clean volumetric flasks. The resulting liquid is a clear pale green color. The flasks are shaken momentarily prior to analysis on the GC-MS instrument.

2.2.2 **Liquid Extraction Method**

The current literature had a very limited amount of analysis on liquid kratom products (41, 77, 78). In addition, the extraction method varied greatly between the sources in terms of the amount of liquid sample used compared to the extraction solvent. In order to make the procedure more simple and in line with the powder extraction methods, a 1:10 sample in 80% MeOH method was tested. All five of the liquid samples that were purchased were tested due to the variation in the products. These included O.P.M.S. Liquid Kratom, Raw Kratom Private Reserve Mitragyna speciosa Extract, Liquid K, Bitang Speciosa Shot, and Zen Liquid Kratom. Three samples were made from each of the kratom products using 1 mL of the product and 9 mL of MeOH in a 10 mL volumetric flask. These were all vortexed for 1 minute and filtered using filter paper into new clean flasks. This produced 15 samples that were tested via GC-MS.

Peaks for both MG and 7OH-MG were seen in all of the samples tested. This indicated that this method could be used on the liquid samples in determining MG and 7OH-MG content.

2.2.3 **O.P.M.S. Liquid Kratom Precipitate**

The O.P.M.S. Liquid Kratom required further analysis. This was because a precipitate formed when the MeOH was added to the sample. In order to ensure that this precipitate was not the compound of interest and to ensure that it was not removing any of this compound when filtered, the precipitate was analyzed.

First, the precipitate was allowed to air dry on the filter paper. It had a brown color and was a fine powder. The powder was introduced to five different solvents for analysis. These included acetonitrile, 100% methanol, methylene chloride, the liquid chromatography mobile phase (95:10 acetonitrile:water), and DMSO. Only methylene chloride (DCM) and DMSO

appeared to dissolve any of the precipitate, but not completely. These were filtered into new flasks to remove the excess precipitate.

The DMSO and DCM liquids were analyzed via GC-MS since they were the only two that appeared to have any effect on the precipitate. However, analysis showed that no MG or 7OH-MG were present nor were any of the other alkaloids associated with kratom. The analysis also indicated that the concentration of anything that did dissolve in the solvents was too low for detection by GC-MS. Due to this, it was determined that the precipitate would not have a significant effect on the analysis for this study.

2.2.4 **Gas Chromatography Mass Spectrometry**

For analysis on the GC-MS instrument, samples were originally analyzed using the current SWGDRUG method (63, 78). This method uses a DB-5 MS column which is 30 m x 0.25 mm x 0.25 μm. The carrier gas used is helium at a 1 mL/min flow rate. The injector is kept at 280 °C; MSD transfer line at 280 °C; MS source at 230 °C; and MS quad at 150 °C. The oven is programmed to start at 100 °C and hold for 2 minutes. This is then brought up at 14 °C/min to 300 °C (~14.25 minutes). This final temperature is held for 25 minutes. The split ratio is 20:1 with a 1 μL injection. The MS scan range is set at 34-550 amu with a threshold of 90. This method had a run time of about 40 minutes.

This SWGDRUG method is used for qualitative purposes. When used on the extracted samples, it was able to detect MG, but the resolution and overall abundance of MG was very poor with only about 550 amu in the extracted ions (figure 3). Because of this the method was changed to see if the method could be shortened in time with a better resolution and ability to quantify.

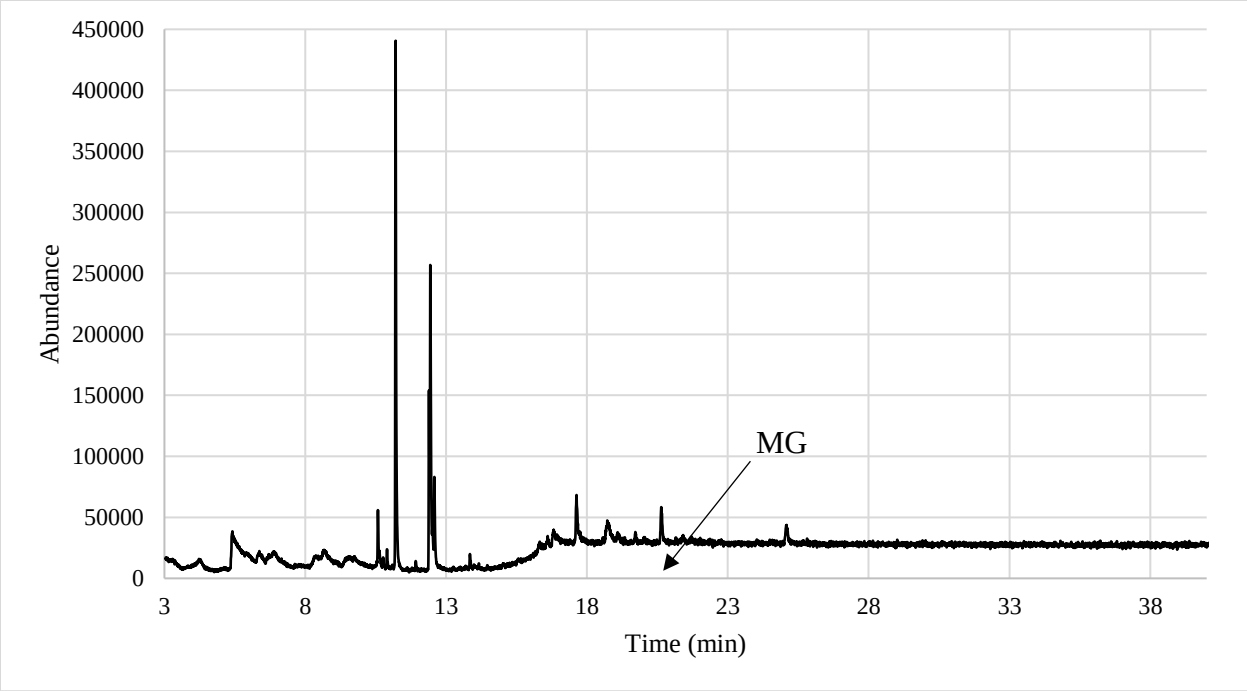

Figure 3. Representative total ion chromatogram of SWGDRUG method with MG peak labeled.

Due to the low response of the instrumentation, the injection volume was changed from 1 µL to 5 µL. This helped increase the response of the instrumentation without overloading it. However, the resolution and response was still low and would not allow for quantification.

One of the key pieces of data noted from the SWGDRUG method included the retention time, which was around 21-22 minutes. At this time, the oven was already holding max temperature. There also did not appear to be any response from injected samples prior to this time other than background noise. Therefore, the next method used increased the starting temperature to 150 °C as well as increasing the temperature ramp from 14 °C/min to 20 °C/min. The final hold at 300 °C was also only held for 10 minutes. This reduced the run time to about 20 minutes. However, this method also did not increase the resolution.

To make the method even more efficient, the start temperature was changed to 250 °C. This was possible due to the fact that the retention of the analytes was still only showing after the temperature had reached 300 °C and was being held. The temperate ramp was also increased to 30 °C/minute. The final hold at 300 °C was reduced to 8.33 minutes. This created a method that had a 10 minute run time. This method provided great resolution for MG as well as 7OH-MG (the main analytes of interest). In addition, 4 other major alkaloids were able to be detected (Figure 4).

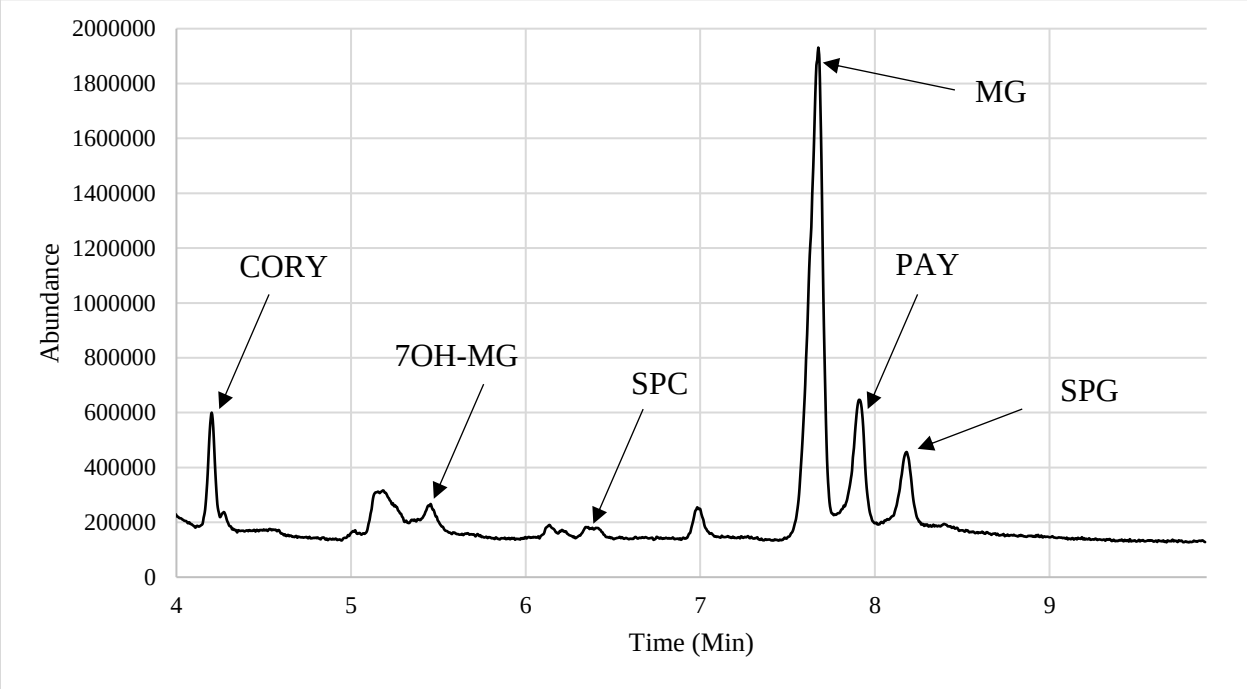

Figure 4. Representative total ion chromatogram of final method with MG, 7OH-MG, PAY peaks labeled as well as CORY, SPC, and SPG

MG, 7OH-MG, PAY, CORY, SPC, and SPG were all determined based on their mass spectrum and reference to current literature (63, 76, 79, 81). Mass spectrum from this study are provided in appendix A for reference.

2.2.5 **Extraction Solvent Analysis**

During this preliminary analysis and method development, it was noted that the GC-MS instrument would need frequent cleaning in order to give accurate results. It was thought that the water present in the 80% MeOH may be contributing a great deal to this issue. To determine if a 100% MeOH solvent could instead be used, the remarkable herbs Vietnam Kratom powder was used. Just as before, 3 samples of 0.1g kratom was sonicated for 30 minutes with 10mL of 100% MeOH. These samples were filtered and then analyzed on the GC-MS. The response given on the instrument showed no difference between the 80% and 100% MeOH. The examination of the instrument showed a benefit to the use of only 100% MeOH as the liner was less dirty than when injecting the 80% MeOH in water. Due to this, the 100% MeOH was decided upon for the actual study.

2.3 **Final Method**

2.3.1 **Powder Product Extraction Method**

The method for extraction from the 22 powder samples used approximately 0.1 g of powder with 10 mL of 100% MeOH. These were done in triplicate from each of the purchased samples. These samples were vortexed for 1 minute followed by a 30 minute sonication at room temperature. Upon completion of the sonication, all samples were filtered into clean glassware

through filter paper. Representative portions of these samples were transferred into GC vials for analysis on the GC-MS instrument.

2.3.2 **Capsule Product Extraction Method**

The 5 capsule samples that were purchased all contained kratom powder. To analyze this powder, the same extraction method as in 2.3.1 was used. However, since there were multiple capsules in each purchased product, three separate capsules were used from each product to provide the three samples. This was done instead of combining all the capsules in order to see if there were any discrepancies in content among the capsules.

2.3.3 **Liquid Product Sample Extraction**

The 5 liquid product samples were first vortexed for 1 minute to ensure uniform distribution of the liquid products. Then 1 mL of sample was combined with 9 mL of 100% MeOH. These were done in triplicate for all samples. These samples were vortexed for 1 minute followed by a 30 minute sonication at room temperature. Upon completion of the sonication, all samples were filtered into clean glassware through filter paper.

The preliminary analysis on liquid samples indicated that the MG concentration of these samples was much higher than that of the powder samples in some of the liquids. In order to quantify these samples, they had to be further diluted with 100% MeOH. The Raw Kratom Private Reserve Extract was diluted using 500 μL of extract with 500 μL of MeOH. The Zen Liquid Kratom and O.P.M.S. Liquid Kratom extracts were diluted by using 250 μL of sample with 750 μL of MeOH. The remaining 2 liquid products did not need dilution due to their preliminary concentrations.

Representative portions of these samples were transferred into GC vials for analysis on the GC-MS instrument.

2.3.4 **Gas Chromatography Mass Spectrometry**

The final method for GC-MS analysis used a DB-5 MS column which is 30 m x 0.25 mm x 0.25 μm. The carrier gas used is helium at a 1 mL/min flow rate. The injector was kept at 280 °C; MSD transfer line at 280 °C; MS source at 230 °C; and MS quad at 150 °C. The oven was programmed to start at 250 °C and immediately increase at 30 °C/minute until achieving 300 °C. This temperature was held for 8.33 minutes giving a total run time of 10 minutes. The samples were injected in pulsed splitless mode with a 5 μL injection volume. The MS scan range was set at 34-550 amu with a threshold of 90. As noted prior, this gave the resolution and response needed to see the major alkaloids present in the kratom samples and allow for quantification.

2.3.5 **Sample Labeling**

Samples were labeled throughout the testing process to avoid confusion during filtering and transferring. They were labeled according to tables II, III and IV which represent the powder, capsule, and liquid samples, respectively.

TABLE II

POWDER SAMPLE LABELING

Brand	Labeling	Type	Label
Kratom One	Tri Force	Powder	KOTP01-03
Kratom One	Red Horn	Powder	KORHP01-03
Kratom One	Bali	Powder	KOBaliP01-03
O.P.M.S. Silver	Malay Special Reserve	Powder	OSMSP01-03
No brand	Red Borneo	Powder	NBRBP01-03
No Brand	Green Malay	Powder	NBGMP01-03
Mr. Nice Guy	Red Sumatra	Powder	NGRSP01-03
Mr. Nice Guy	Red Gold	Powder	NGRGP01-03
Mr. Nice Guy	Red Bali	Powder	NGRBaliP01-03
Mr. Nice Guy	Red Borneo	Powder	NGRBP01-03
Mr. Nice Guy	Green Bali	Powder	NGGBaliP01-03
Mr. Nice Guy	Green Sumatra	Powder	NGGSP01-03
Mr. Nice Guy	Green Vietnam	Powder	NGGVP01-03
Mr. Nice Guy	White Borneo	Powder	NGWBP01-03
Mr. Nice Guy	White Bali	Powder	NGWBaliP01-03
Mr. Nice Guy	White Gold	Powder	NGWGP01-03
Remarkable Herbs	Indo	Powder	RHIP01-03
Remarkable Herbs	Bali	Powder	RHBaliP01-03
Remarkable Herbs	Thai	Powder	RHTP01-03
Remarkable Herbs	Maeng Da	Powder	RHMaP01-03
Remarkable Herbs	Malaysian	Powder	RHMP01-03
Remarkable Herbs	Vietnam	Powder	RHVP01-03

TABLE III

CAPSULE SAMPLE LABELING

Brand	Labeling	Type	Label
Tropical Kratom	Green Strain	Capsule	TKGSC01-03
Tropical Kratom	White Strain	Capsule	TKWSC01-03
No Brand	Bali	Capsule	NBBC01-03
Choice Kratom	Maeng Da	Capsule	CKMC01-03
Kratom Kaps	Indo	Capsule	KKIC01-03

TABLE IV

LIQUID SAMPLE LABELING

Brand	Labeling	Type	Label
O.P.M.S.	Liquid Kratom	Liquid	OPLKL01-03
Bintang	Speciosa Shot	Liquid	BSSL01-03
Liquid K	N/A	Liquid	LKSL01-03
Raw Kratom	Private Reserve Mitragyna Speciosa Extract	Liquid	RKPRL01-03
Zen	Liquid Kratom	Liquid	ZLKL01-03

2.3.6 **Creation of Standards**

For all standards, a signal to noise ratio of 10:1 was used for the limit of quantitation (LOQ) and 3:1 for the limit of detection (LOD). All standards were created using 100% MeOH. Due to the amount of standard purchased as well as to avoid any resolution issues on instrumentation, separate standards were made for MG, 7OH-MG, and PAY.

The MG concentrations used for a quantitative standard curve included 0.01 mg/mL (LOD), 0.02 mg/mL (LOQ), 0.05 mg/mL, 0.10 mg/mL, 0.25 mg/mL, 0.50 mg/mL and 0.75 mg/mL.

The 7OH-MG concentrations used for a quantitative standard curve included 0.01 mg/mL (LOD), 0.02 mg/mL (LOQ), 0.05 mg/mL, 0.10 mg/mL, 0.25 mg/mL, and 0.50 mg/mL. The limited amount of standard (1 mg) was the reason for the high concentration stopping at 0.50 mg/mL. In addition, during preliminary analysis it was noted that the 7OH-MG peak was much less pronounced than that of MG indicating its concentration was much lower relatively.

The PAY concentrations used for a quantitative standard curve included 0.01 mg/mL (LOD), 0.02 mg/mL (LOQ), 0.05 mg/mL, 0.10 mg/mL, 0.25 mg/mL, and 0.50 mg/mL. For similar reasons as 7OH-MG, the highest concentration selected was 0.50 mg/mL.

3. RESULTS

3.1 Standard Curve

It was expected that the standard curve for MG, 7OH-MG, and PAY would result in a linear curve. However, they instead resulted in a quadratic curve that is represented by the following equation:

$$Y=Ax^2+Bx-C$$

All standard curves had an R^2 value of ≥ 0.99. The chosen LOD of 0.1 mg/mL for all compounds was appropriate based on the 3:1 signal to noise ratio. The chosen LOQ of 0.20 mg/mL for all compounds was also appropriate based on the 10:1 signal to noise ratio. A representative curve for MG, 7OH-MG, and PAY can be seen in figures 5, 6, and 7 respectively.

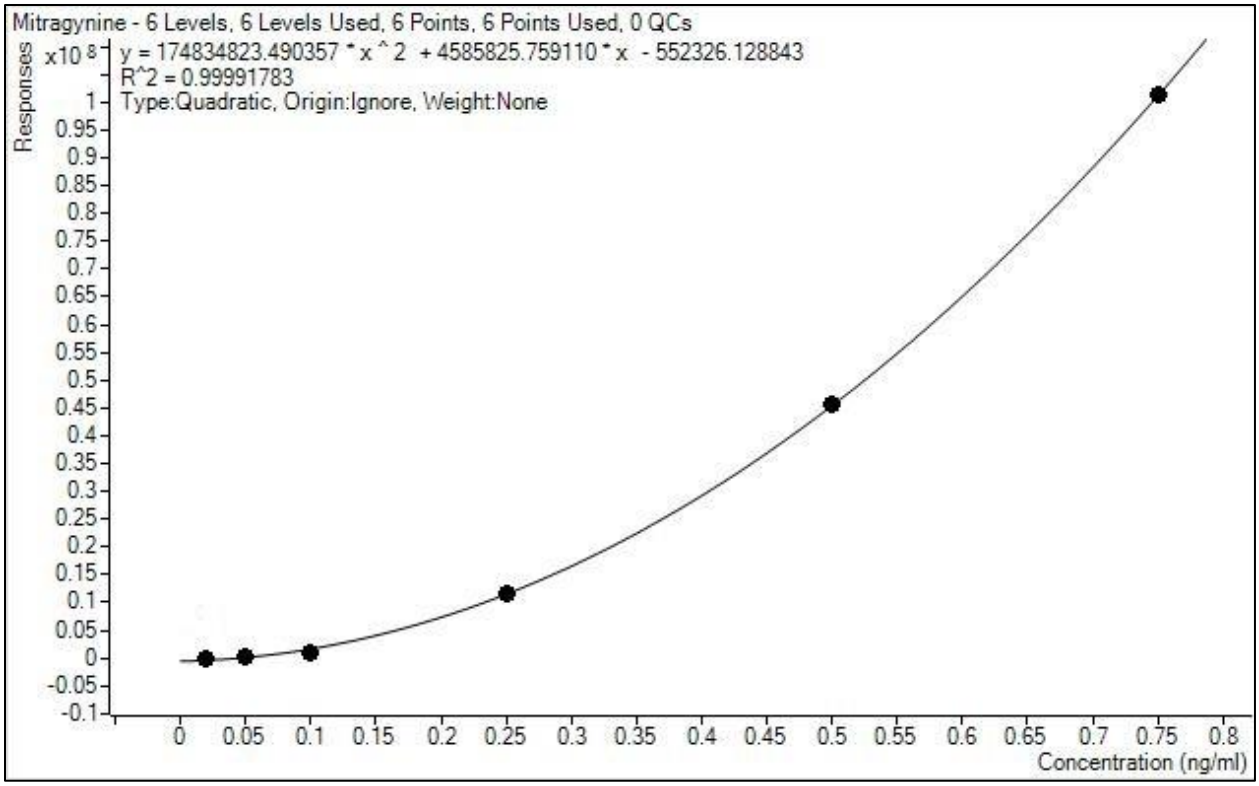

Figure 5. Standard curve of MG using the concentrations 0.02 (LOQ), 0.05, 0.1, 0.25, 0.5, and 0.75 mg/mL from the MassHunter software.

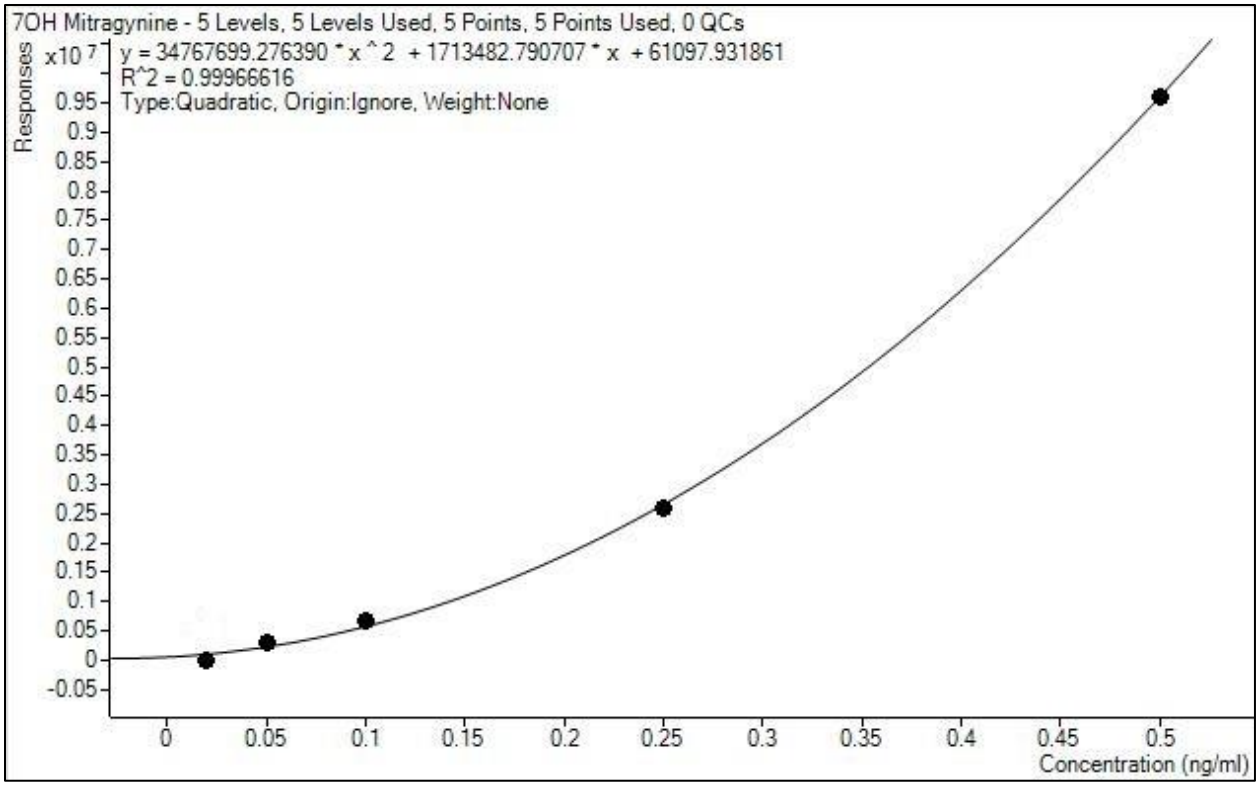

Figure 6. Standard curve of 7OH-MG using the concentrations 0.02 (LOQ), 0.05, 0.1, 0.25, and 0.5 mg/mL from the MassHunter software.

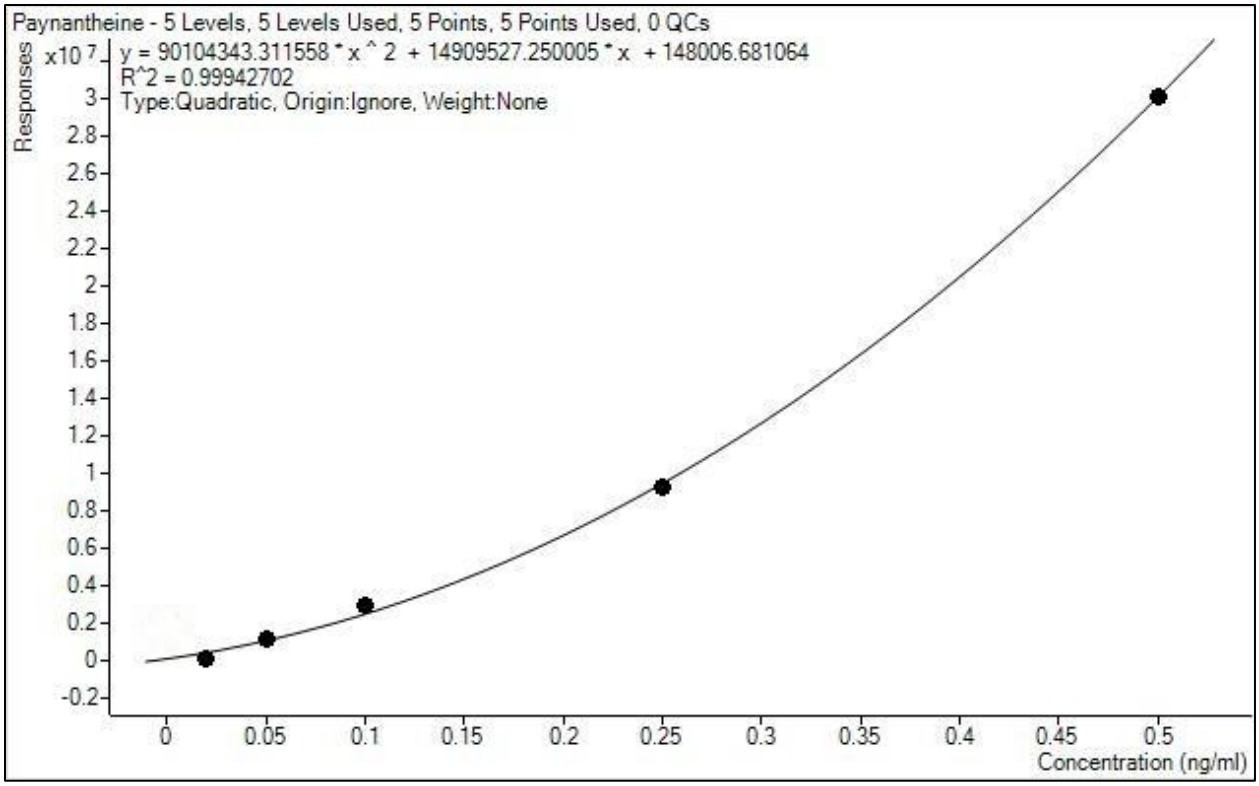

Figure 7. Standard curve of PAY using the concentrations 0.02 (LOQ), 0.05, 0.1, 0.25, and 0.5 mg/mL from the MassHunter software.

3.2 **Product Results**

3.2.1 **Mitragynine**

MG was detected in all 32 samples analyzed indicating that these products are indeed kratom. In 30 of these samples, the amount of MG present could be quantitated. The 2 samples that did not meet the LOQ did meet the LOD criteria. Both samples were liquid products.

The amount of MG present in the powder samples ranged from 3.26 to 12.56 mg/g. The amount of MG present in the capsule samples ranged from 3.30 to 3.55 mg/g. The amount of

MG present in the liquid samples ranged from 3.25 to 6.87 mg/mL. The results are summarized in table V and VI. All calculations and data for MG can be found in appendix B.

TABLE V

MITRAGYNINE CONCENTRAION OF POWDER AND CAPSULE SAMPLES

Brand	Labeling	Type	Concentration (mg/g)
Kratom One	Tri Force	Powder	3.26
Kratom One	Red Horn	Powder	3.27
Kratom One	Bali	Powder	6.40
O.P.M.S. Silver	Malay Special Reserve	Powder	8.80
No Brand	Red Borneo	Powder	6.05
No Brand	Green Malay	Powder	8.66
Mr. Nice Guy	Red Sumatra	Powder	7.07
Mr. Nice Guy	Red Gold	Powder	12.56
Mr. Nice Guy	Red Bali	Powder	6.46
Mr. Nice Guy	Red Borneo	Powder	7.94
Mr. Nice Guy	Green Bali	Powder	5.81
Mr. Nice Guy	Green Sumatra	Powder	10.60
Mr. Nice Guy	Green Vietnam	Powder	7.85
Mr. Nice Guy	White Borneo	Powder	6.15
Mr. Nice Guy	White Bali	Powder	4.20
Mr. Nice Guy	White Gold	Powder	7.14
Remarkable Herbs	Indo	Powder	8.80
Remarkable Herbs	Bali	Powder	7.31
Remarkable Herbs	Thai	Powder	10.43
Remarkable Herbs	Maeng Da	Powder	9.07
Remarkable Herbs	Malaysian	Powder	9.77
Remarkable Herbs	Vietnam	Powder	6.64
Tropical Kratom	Green Strain	Capsule	3.55
Tropical Kratom	White Strain	Capsule	3.38
No Brand	Bali	Capsule	3.35
Choice Kratom	Maeng Da	Capsule	3.30
Kratom Kaps	Indo	Capsule	3.31

TABLE VI

MITRAGYNINE CONCENTRAION OF LIQUID SAMPLES

Brand	Labeling	Concentration (mg/mL)	Total Content (mg)
O.P.M.S.	Liquid Kratom	6.87	54.95
Bintang	Speciosa Shot	Detected	N/A
Liquid K	N/A	Detected	N/A
Raw Kratom	Private Reserve Mitragyna Speciosa Extract	3.25	16.25
Zen	Liquid Kratom	5.39	43.14

3.2.2 **7-hydroxymitragynine**

7OH-MG was detected in 13 of the 32 samples analyzed. Only 7 of these samples had levels high enough to meet the quantitation criteria. 6 of the samples met the LOD criteria. The remaining 19 samples did not meet the LOQ or the LOD criteria.

The amount of 7OH-MG present in the powder samples could only be quantitated in 2 samples and was 2.02 mg/g. 2 other powder samples met the LOD criteria. The amount of 7OH-MG present in the capsule samples ranged from 2.02 to 2.03 mg/g. The amount of 7OH-MG present in the liquid samples could only be quantified in one sample and was 0.46 mg/mL. The results are summarized in table VII and VIII. All calculations and data for 7OH-MG can be found in appendix C.

TABLE VII

7-HYDROXYMITRAGYNINE CONCENTRAION OF POWDER AND CAPSULE SAMPLES

Brand	Labeling	Type	Concentration (mg/g)
Kratom One	Tri Force	Powder	2.03
Kratom One	Red Horn	Powder	2.02
Kratom One	Bali	Powder	-
O.P.M.S. Silver	Malay Special Reserve	Powder	-
No Brand	Red Borneo	Powder	-
No Brand	Green Malay	Powder	-
Mr. Nice Guy	Red Sumatra	Powder	-
Mr. Nice Guy	Red Gold	Powder	-
Mr. Nice Guy	Red Bali	Powder	-
Mr. Nice Guy	Red Borneo	Powder	-
Mr. Nice Guy	Green Bali	Powder	-
Mr. Nice Guy	Green Sumatra	Powder	-
Mr. Nice Guy	Green Vietnam	Powder	-
Mr. Nice Guy	White Borneo	Powder	-
Mr. Nice Guy	White Bali	Powder	Detected
Mr. Nice Guy	White Gold	Powder	-
Remarkable Herbs	Indo	Powder	-
Remarkable Herbs	Bali	Powder	Detected
Remarkable Herbs	Thai	Powder	-
Remarkable Herbs	Maeng Da	Powder	-
Remarkable Herbs	Malaysian	Powder	-
Remarkable Herbs	Vietnam	Powder	-
Tropical Kratom	Green Strain	Capsule	2.03
Tropical Kratom	White Strain	Capsule	2.03
No Brand	Bali	Capsule	Detected
Choice Kratom	Maeng Da	Capsule	Detected
Kratom Kaps	Indo	Capsule	2.03

TABLE VIII

7-HYDROXYMITRAGYNINE CONCENTRAION OF LIQUID SAMPLES

Brand	Labeling	Concentration (mg/mL)	Total Content (mg)
O.P.M.S.	Liquid Kratom	Detected	-
Bintang	Speciosa Shot	Detected	-
Liquid K	N/A	Detected	-
Raw Kratom	Private Reserve Mitragyna Speciosa Extract	0.47	2.34
Zen	Liquid Kratom	Detected	-

3.2.3 **Paynantheine**

PAY was detected in 30 of the 32 samples analyzed. Of these 30 samples, 7 did not meet the LOQ criteria but did meet the LOD criteria. 2 samples did not meet the LOD or LOQ criteria.

The amount of PAY present in the powder samples ranged from 2.38 to 6.77 mg/g. The amount of PAY present in the capsule samples could not be quantified, but all 5 samples did meet the LOD. The amount of PAY present in the liquid samples was quantified with 3 of the samples and ranged from 0.91 to 1.96 mg/mL. The remaining 2 liquid samples did not meet the LOD criteria. The results are summarized in tables IX and X. All calculations and data for PAY can be found in appendix D.

TABLE IX

PAYNANTHEINE CONCENTRAION OF POWDER AND CAPSULE SAMPLES

Brand	Labeling	Type	Concentration (mg/g)
Kratom One	Tri Force	Powder	Detected
Kratom One	Red Horn	Powder	Detected
Kratom One	Bali	Powder	5.62
O.P.M.S. Silver	Malay Special Reserve	Powder	3.27
No Brand	Red Borneo	Powder	3.18
No Brand	Green Malay	Powder	2.78
Mr. Nice Guy	Red Sumatra	Powder	2.45
Mr. Nice Guy	Red Gold	Powder	4.42
Mr. Nice Guy	Red Bali	Powder	5.98
Mr. Nice Guy	Red Borneo	Powder	6.04
Mr. Nice Guy	Green Bali	Powder	5.33
Mr. Nice Guy	Green Sumatra	Powder	3.51
Mr. Nice Guy	Green Vietnam	Powder	5.37
Mr. Nice Guy	White Borneo	Powder	3.69
Mr. Nice Guy	White Bali	Powder	3.87
Mr. Nice Guy	White Gold	Powder	2.38
Remarkable Herbs	Indo	Powder	2.79
Remarkable Herbs	Bali	Powder	6.77
Remarkable Herbs	Thai	Powder	3.17
Remarkable Herbs	Maeng Da	Powder	3.02
Remarkable Herbs	Malaysian	Powder	3.38
Remarkable Herbs	Vietnam	Powder	4.19
Tropical Kratom	Green Strain	Capsule	Detected
Tropical Kratom	White Strain	Capsule	Detected
No Brand	Bali	Capsule	Detected
Choice Kratom	Maeng Da	Capsule	Detected
Kratom Kaps	Indo	Capsule	Detected

TABLE X

7-HYDROXYMITRAGYNINE CONCENTRAION OF LIQUID SAMPLES

Brand	Labeling	Concentration (mg/mL)	Total Content (mg)
O.P.M.S.	Liquid Kratom	1.96	15.68
Bintang	Speciosa Shot	-	-
Liquid K	N/A	-	-
Raw Kratom	Private Reserve Mitragyna Speciosa Extract	0.91	4.56
Zen	Liquid Kratom	1.20	9.57

4. DISCUSSION

4.1 Comparison to Reported Concentrations

Of the 32 samples, 6 of the powder samples and 1 of the liquid samples had concentrations written on the packaging. All of the samples had calculated levels of MG lower than what was reported on the packaging. These results are summarized in table XI. Example calculations can be seen in appendix B. These samples also had reported concentrations of 7OH-MG but the levels were below the LOQ and could not be compared. It is unknown how the companies calculate their MG and 7OH-MG content.

TABLE XI

CALCULATED VERSUS REPORTED CONCENTRATION OF MITRAGYNINE

Brand	Labeling	Type	Calculated Content	Reported Content
O.P.M.S.	Liquid Kratom	Liquid	54.95 mg	95mg
Remarkable Herbs	Indo	Powder	0.88%	1.50%
Remarkable Herbs	Bali	Powder	0.73%	1.25%
Remarkable Herbs	Thai	Powder	1.04%	1.30%
Remarkable Herbs	Maeng Da	Powder	0.91%	1.10%
Remarkable Herbs	Malaysian	Powder	0.97%	1.55%
Remarkable Herbs	Vietnam	Powder	0.66%	1.50%

It should be noted that the powder products labeled with the highest amount of MG – Remarkable Herbs Malaysian, Indo, and Vietnam Kratom – actually did not have the highest concentration of MG found. The Vietnam product actually contained the least amount of MG

which was only 40% of what was reported on the label. The Malaysian and Indo products only contained 63% and 59% of the reported concentration, respectively.

In fact, it was found that the powder product labeled with the lowest amount of MG – Remarkable Herbs Maeng Da – had a higher concentration than both the Vietnam and the Indo products. The highest concentration found was actually in the Remarkable Herbs Thai product which was labeled as the middle of the concentrations at 1.30%. All of the products had concentrations calculated to be less than what was reported on the label.

4.2 **Comparison Between Brands**

Many of the brands purchased had labeling that would indicate they were similar to each other. For example, the brand Remarkable Herbs had a kratom product labeled bali that could be similar to the Mr. Nice Guy brand of red bali, green bali, and/or white bali. Similarly, products were labeled as vietnam, borneo, malaysian, sumatra, and gold kratom. However, it was found that there was a variation in the MG content between brands of the same type of labeling (figure 8). The bali products were shown to have a concentration ranging from 4.20 to 7.31 mg/g and the borneo products were shown to have a concentration of 6.05 to 7.94 mg/g.

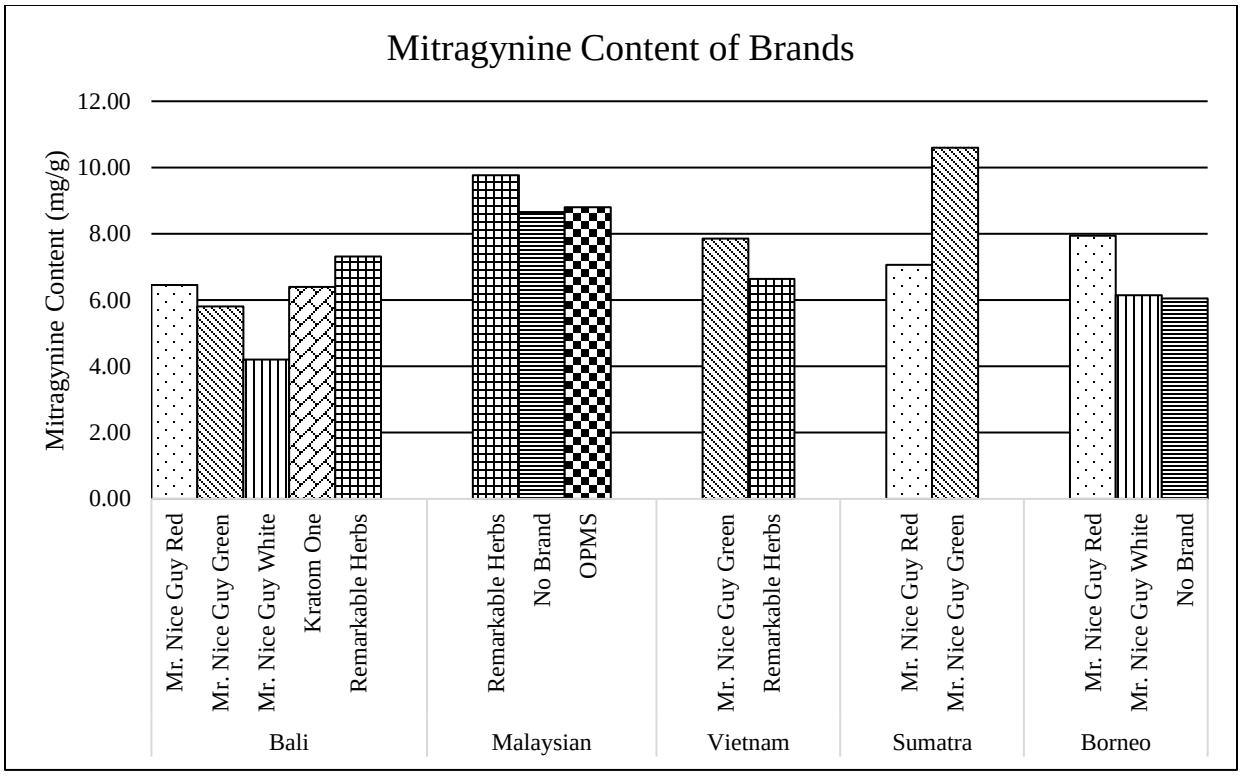

Figure 8. Mitragynine content of the types of kratom (Bali, Malaysian, Vietnam, etc.) compared across brands

It was also found that MG content within the same brand varied depending on the labeling (figure 9). For example, the Remarkable Herbs brand had a range of 6.64 to 10.43 mg/g of MG while the Mr. Nice Guy brand had a range of 4.20-12.56 mg/g of MG.

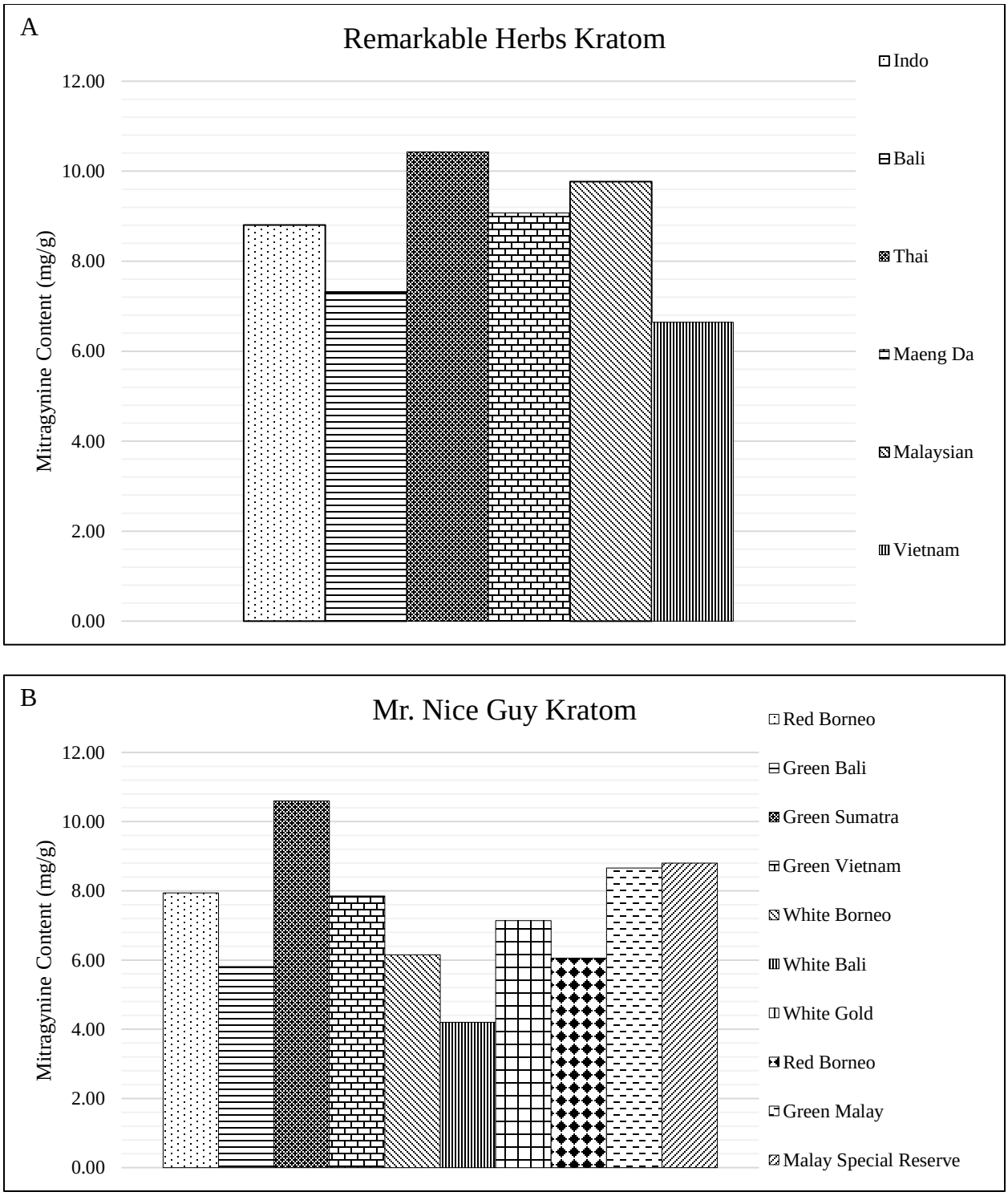

Figure 9. A) Comparison of the MG content of Remarkable Herbs brand kratom products. B) Comparison of the MG content of Mr. Nice Guy kratom products.

In a similar fashion, PAY concentrations varied between and within the brands although at a lower concentration range of 3.18 to 6.04 mg/g. Further figures can be seen in appendix E.

4.3 **Implications**

It is anecdotally reported that certain ‘strains’ of kratom lead to different effects. Red strains are typically associated with calming effects and help alleviate pain; green strains are stimulating and last the longest of the three; and white strains are better at boosting energy and mood levels. Although this study did not focus on the effects of kratom or the different strains, the MG content does not show any trend that would indicate a difference between the three types.

It is also anecdotally reported that users can build tolerance to kratom and could experience withdrawal symptoms. Although not focused on in this study, the variation of 3.26 to 12.56 mg/g of MG could mean that tolerance can be built faster or slower depending on the type used. This could lead to taking a larger amount of kratom in the future which may increase dependency and withdrawal symptoms later. In addition, the higher 12.56 mg/g dose could lead to a stronger response felt as compared to the lower dose of 3.26 mg/g in a new user.

The variation in the concentration of PAY from 3.18 to 6.04 mg/g could have unintended negative effects as well. As previously stated, it is thought that PAY may have psychoactive activity. If it does indeed have psychoactive properties, the higher concentration products in combination with a higher concentration MG product may have negative health consequences.

Although the 7OH-MG concentrations were below the LOQ, a few of the products did show a concentration of 2.02 mg/g. It is reported that this compound could be as much as 30 times as potent as MG. Products that have higher levels of 7OH-MG could lead to faster

dependency and tolerance in the user. Mixing of products that contain high doses of these compounds could make these effects even stronger.

MG, 7OH-MG, and PAY compounds in this study showed great variation between the products. This type of variation could create a dangerous situation if not properly regulated and tested. Kratom needs to be studied further to ensure that the health of the individual is not at risk and that the products being sold are more reliable.

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APPENDECIES

APPENDIX A

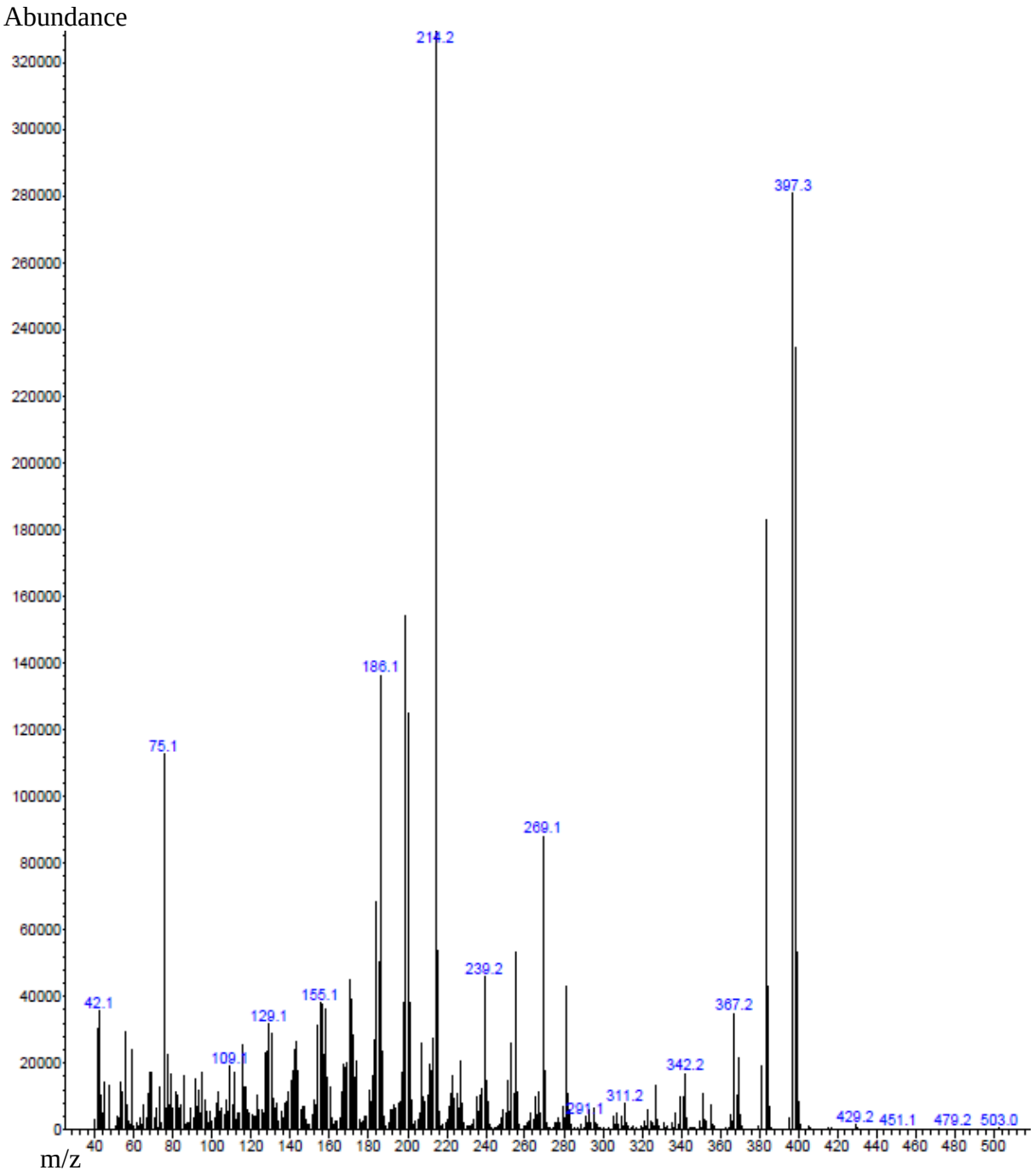

Figure 10. Mass Spectrum of mitragynine.

APPENDIX A (CONT.)

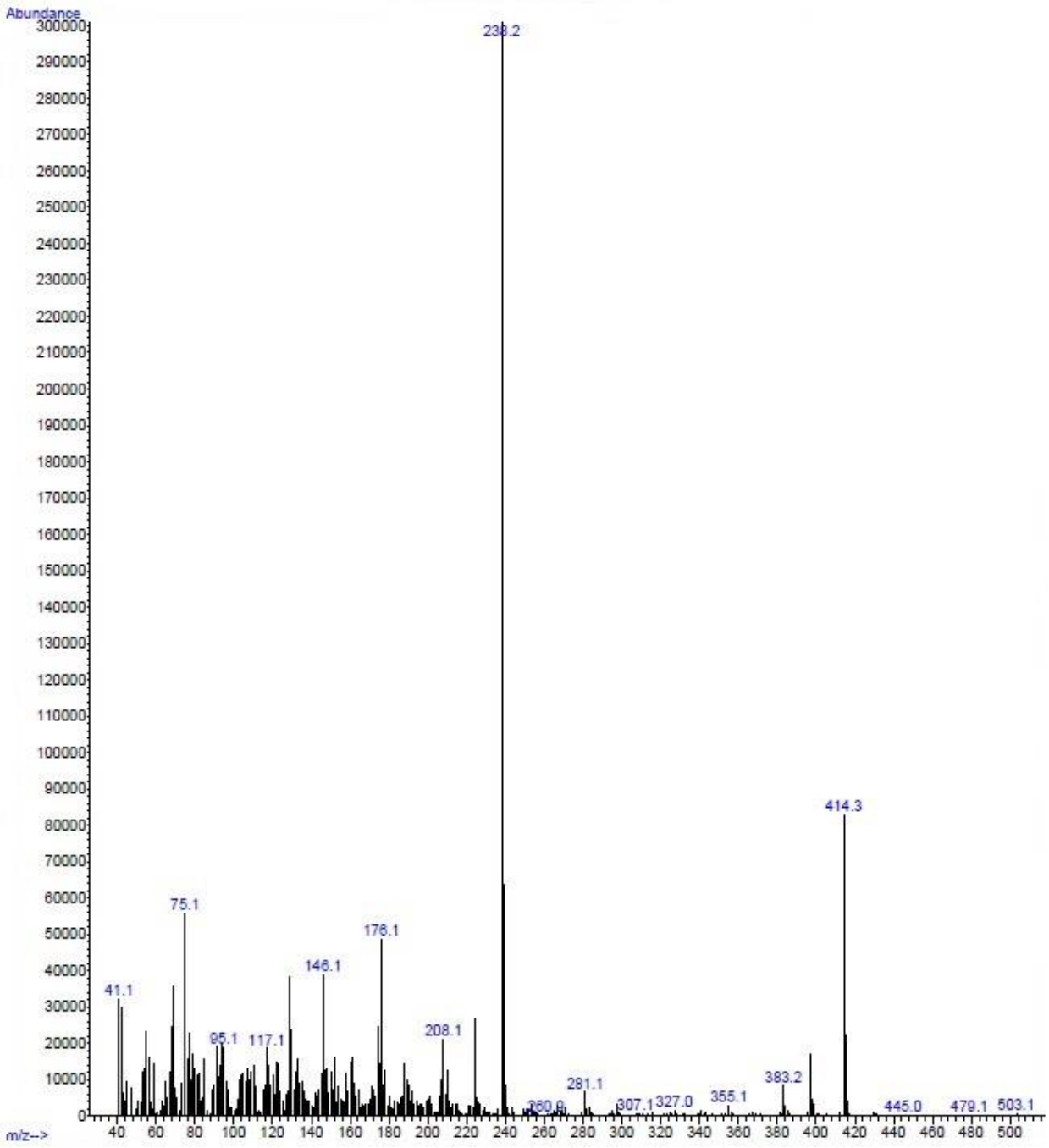

Figure 11. Mass spectrum of 7-hydroxymitragynine.

APPENDIX A (CONT.)

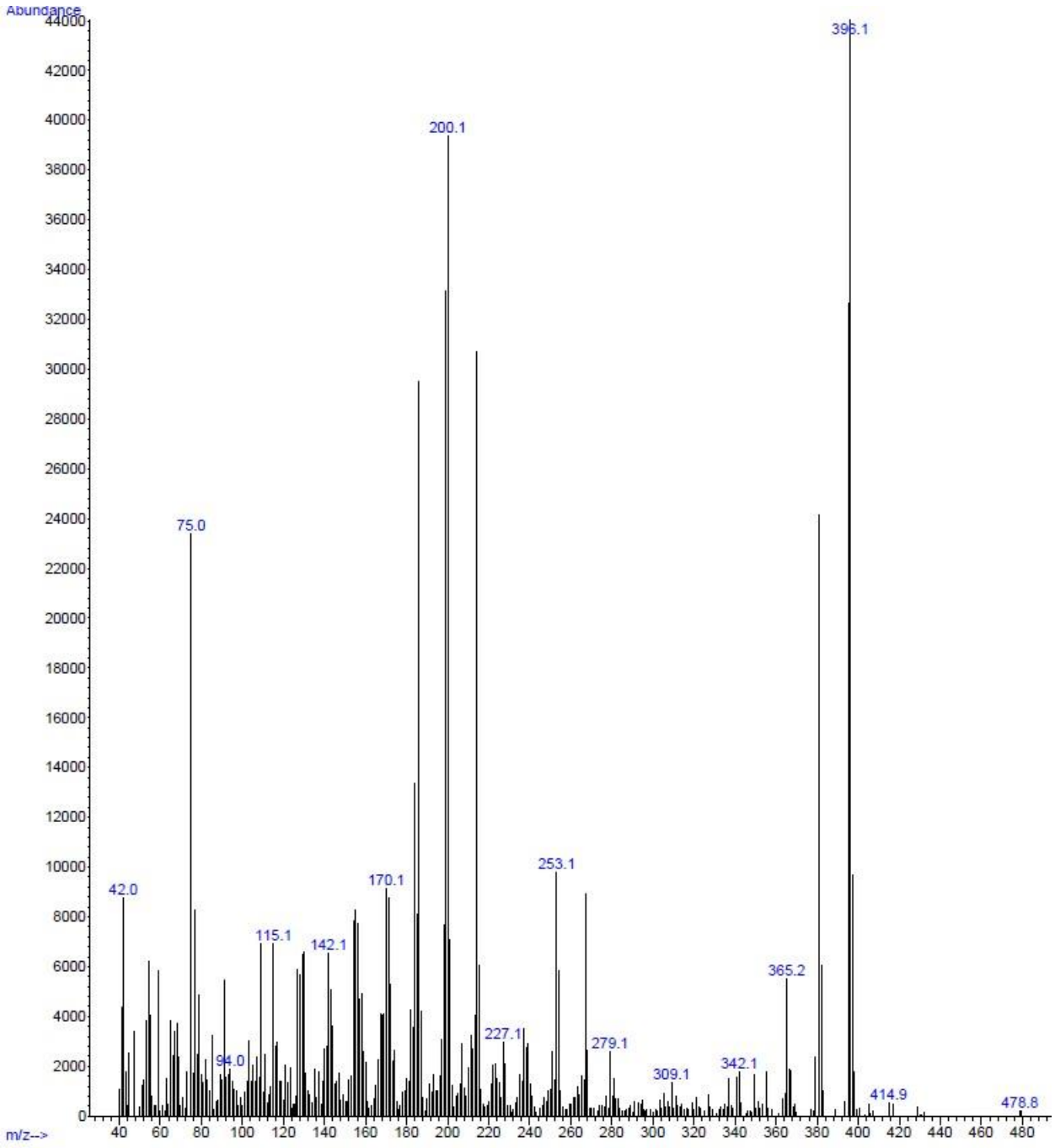

Figure 12. Mass spectrum of paynantheine.

APPENDIX A (CONT.)

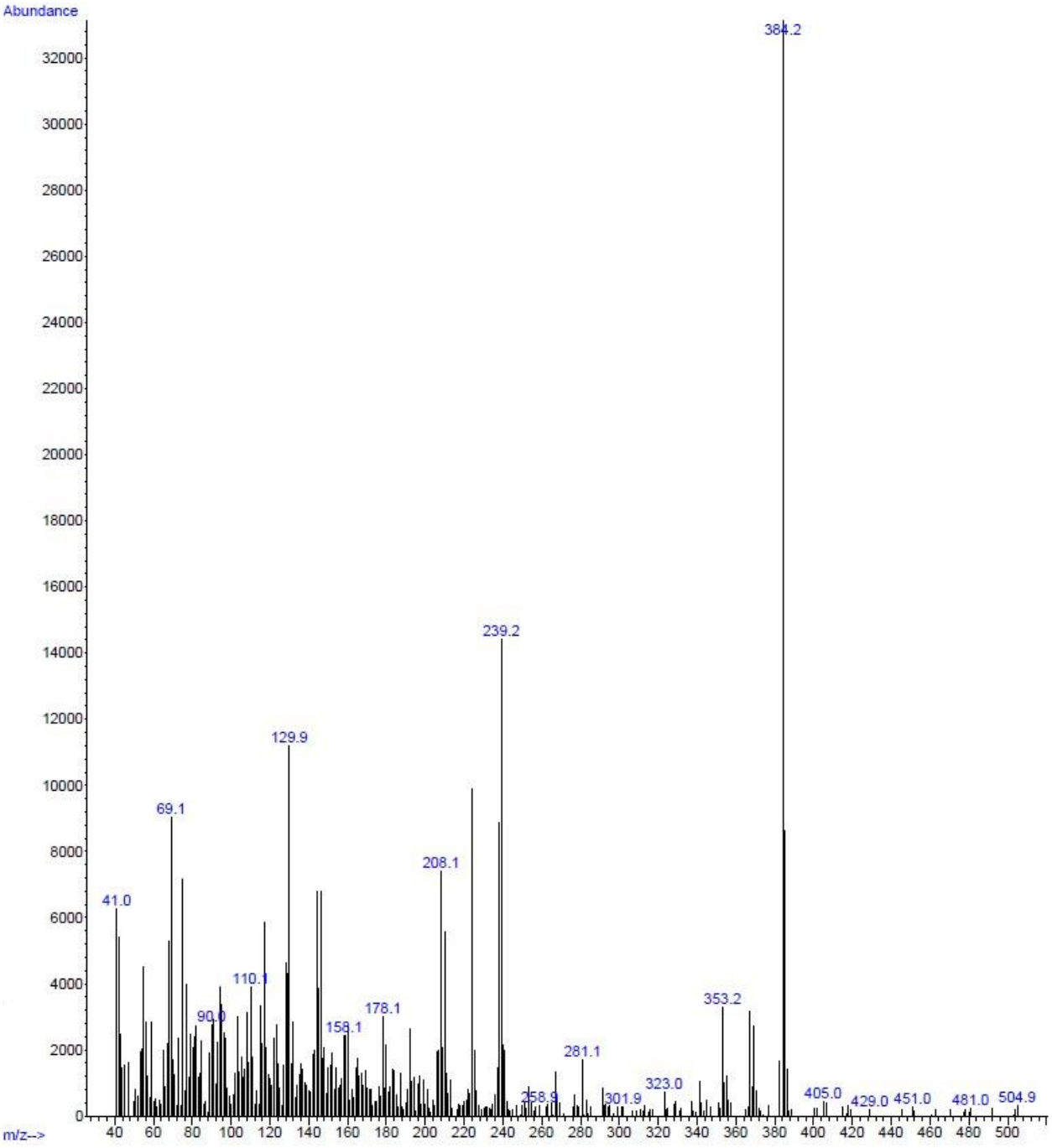

Figure 13. Mass spectrum of corynantheine.

APPENDIX A (CONT.)

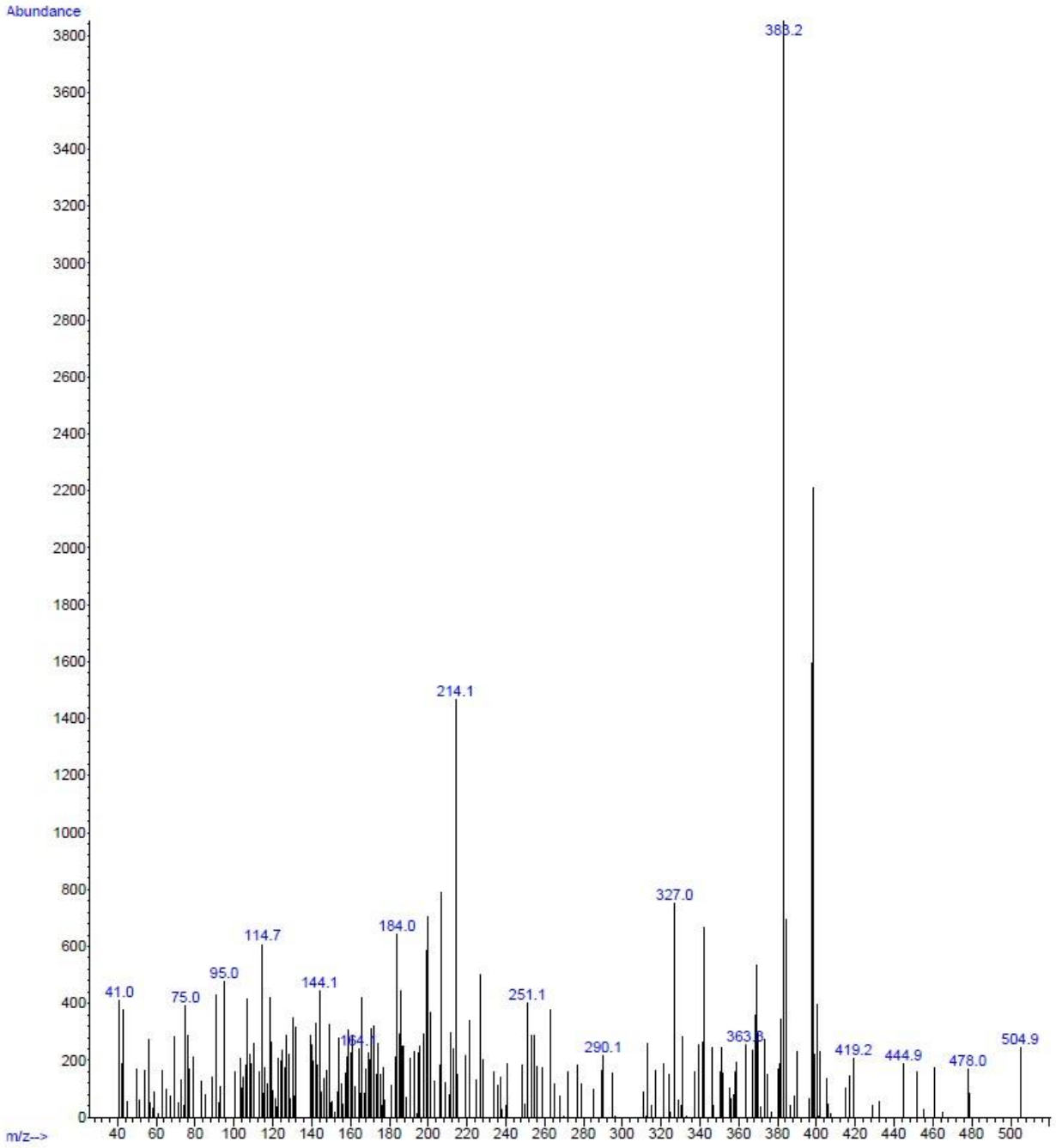

Figure 14. Mass spectrum of speciociliatine.

APPENDIX A (CONT.)

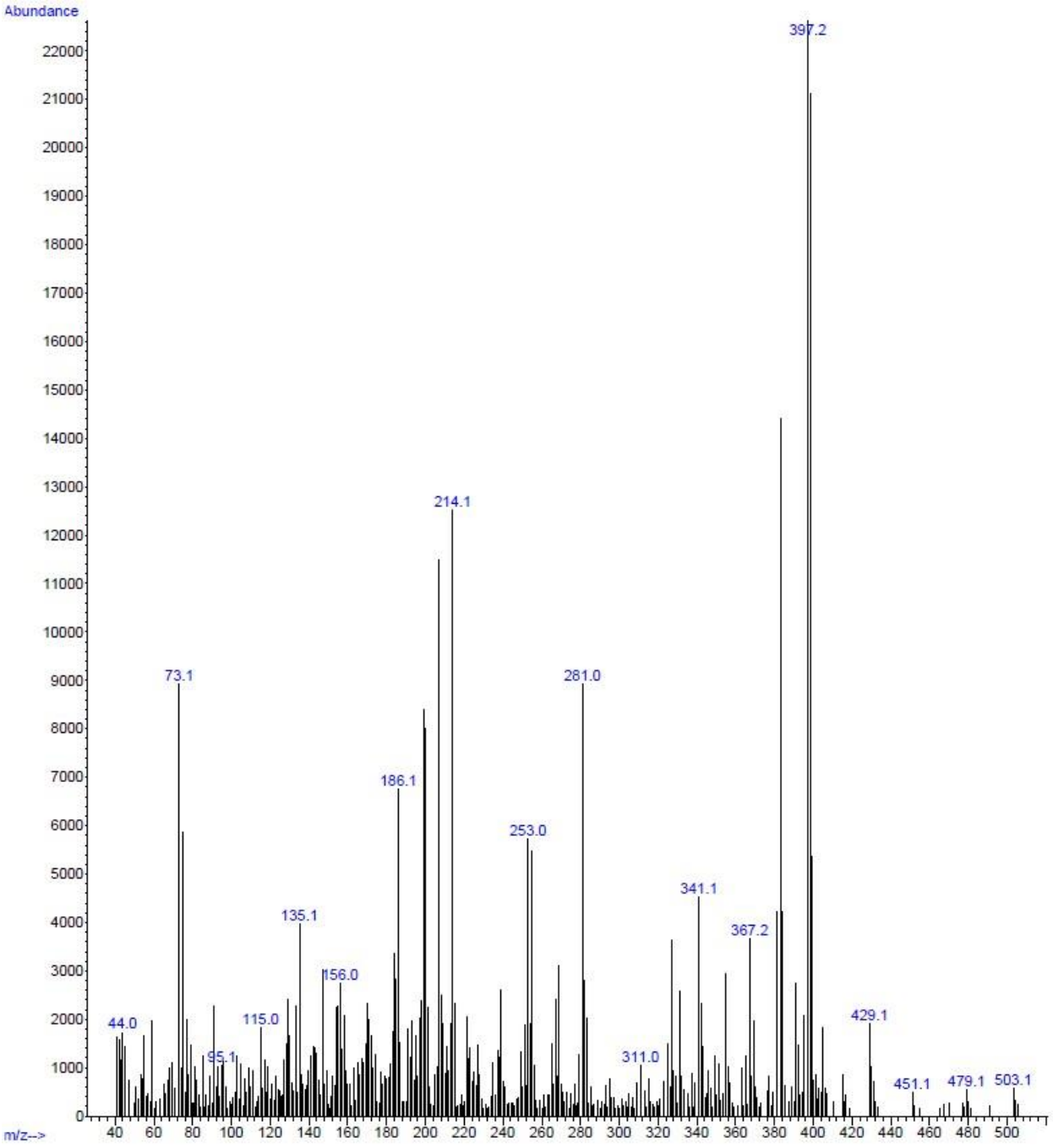

Figure 15. Mass spectrum of speciogynine.

APPENDIX B

Sample Concentrations

Concentrations of samples were done using Agilent Technologies MassHunter Quantitative Analysis Software, Version B.07.01. Sample concentrations were rounded to two decimal places.

Sample MG Percentage

The product's MG percentage was calculated using the following equation:

$$(A \text{ mg/mL} \times 10 \text{ mL}) / B \text{ mg} \times 100 = C$$

Where A is the MG concentration as reported by the software, B is the amount of sample used to create the sample, and C is the final percentage. A was multiplied by 10 to account for the total MG content of the samples since they were prepared in 10 mL of methanol. Using the Remarkable Herbs Vietnam Kratom as an example:

$$(0.065044304 \text{ mg/mL} \times 10 \text{ mL}) / 100.1 \text{ mg} \times 100 = 0.65109413\%$$

Since all products were run in triplicate, this result was averaged when reported. This data can be seen in table XII

APPENDIX B (CONT.)

TABLE XII

MITRAGYNINE PERCENTAGE OF POWDER AND CAPSULE PRODUCTS

Name	Calc. Conc. (mg/mL)	Mass Used (g)	Mass Used (mg)	Total Conc. (mg)	Percentage	AVG %
TKGSC01	0.0333	0.1001	100.1	0.3332	0.3328	0.3551
TKGSC02	0.0354	0.1000	100.0	0.3544	0.3544	
TKGSC03	0.0379	0.1002	100.2	0.3788	0.3780	
TKWSC01	0.0329	0.1000	100.0	0.3287	0.3287	0.3384
TKWSC02	0.0336	0.1001	100.1	0.3360	0.3357	
TKWSC03	0.0351	0.1000	100.0	0.3509	0.3509	
KKIC01	0.0334	0.1000	100.0	0.3340	0.3340	0.3311
KKIC02	0.0327	0.1001	100.1	0.3268	0.3264	
KKIC3	0.0333	0.1000	100.0	0.3330	0.3330	
NBBC01	0.0336	0.0999	99.9	0.3359	0.3363	0.3347
NBBC02	0.0333	0.1000	100.0	0.3328	0.3328	
NBBC03	0.0335	0.1000	100.0	0.3351	0.3351	
CKMaC01	0.0331	0.1002	100.2	0.3314	0.3307	0.3302
CKMaC02	0.0331	0.1001	100.1	0.3314	0.3311	
CKMaC03	0.0329	0.1000	100.0	0.3289	0.3289	
KORHP01	0.0326	0.1000	100.0	0.3263	0.3263	0.3269
KORHP02	0.0328	0.1001	100.1	0.3278	0.3275	
KORHP03	0.0328	0.1002	100.2	0.3278	0.3271	
KOTP01	0.0326	0.0999	99.9	0.3262	0.3265	0.3262
KOTP02	0.0326	0.1000	100.0	0.3263	0.3263	
KOTP03	0.0326	0.1000	100.0	0.3259	0.3259	
RHIP01	0.0886	0.1000	100.0	0.8856	0.8856	0.8803
RHIP02	0.0870	0.1001	100.1	0.8696	0.8687	
RHIP03	0.0886	0.0999	99.9	0.8855	0.8864	
RHTP01	0.1184	0.1002	100.2	1.1841	1.1817	1.0428
RHTP02	0.0986	0.1001	100.1	0.9863	0.9853	
RHTP03	0.0962	0.1001	100.1	0.9624	0.9614	

APPENDIX B (CONT.)

TABLE XII (CONT.)

MITRAGYNINE PERCENTAGE OF POWDER AND CAPSULE PRODUCTS

Name	Calc. Conc. (mg/mL)	Mass Used (g)	Mass Used (mg)	Total Conc. (mg)	Percentage	AVG %
RHMaP01	0.0939	0.0999	99.9	0.9388	0.9398	0.9070
RHMaP02	0.0866	0.1002	100.2	0.8663	0.8646	
RHMaP03	0.0918	0.1002	100.2	0.9185	0.9166	
NGRSP01	0.0650	0.1002	100.2	0.6500	0.6487	0.7067
NGRSP02	0.0762	0.0999	99.9	0.7615	0.7623	
NGRSP03	0.0709	0.1000	100.0	0.7090	0.7090	
NGGSP01	0.0962	0.1001	100.1	0.9617	0.9608	1.0597
NGGSP02	0.1110	0.1001	100.1	1.1105	1.1094	
NGGSP03	0.1109	0.1000	100.0	1.1090	1.1090	
NBGMP01	0.0812	0.1002	100.2	0.8117	0.8101	0.8658
NBGMP02	0.0883	0.1000	100.0	0.8826	0.8826	
NBGMP03	0.0905	0.1000	100.0	0.9046	0.9046	
RHMP01	0.0885	0.1002	100.2	0.8853	0.8836	0.9768
RHMP02	0.0988	0.1001	100.1	0.9882	0.9872	
RHMP03	0.1062	0.1002	100.2	1.0618	1.0597	
OSMSP01	0.0803	0.1002	100.2	0.8030	0.8014	0.8805
OSMSP02	0.0967	0.1000	100.0	0.9673	0.9673	
OSMSP03	0.0874	0.1001	100.1	0.8736	0.8727	
NGRGP01	0.1398	0.1000	100.0	1.3976	1.3976	1.2561
NGRGP02	0.1086	0.1001	100.1	1.0859	1.0849	
NGRGP03	0.1287	0.1001	100.1	1.2870	1.2858	
NGWGP01	0.0724	0.1001	100.1	0.7236	0.7229	0.7139
NGWGP02	0.0707	0.1000	100.0	0.7072	0.7072	
NGWGP03	0.0713	0.1002	100.2	0.7131	0.7117	
NBRBP01	0.0595	0.0999	99.9	0.5950	0.5956	0.6053
NBRBP02	0.0597	0.1001	100.1	0.5972	0.5966	
NBRBP03	0.0624	0.1000	100.0	0.6236	0.6236	

APPENDIX B (CONT.)

TABLE XII (CONT.)

MITRAGYNINE PERCENTAGE OF POWDER AND CAPSULE PRODUCTS

Name	Calc. Conc. (mg/mL)	Mass Used (g)	Mass Used (mg)	Total Conc. (mg)	Percentage	AVG %
NGRBP01	0.0789	0.1002	100.2	0.7888	0.7872	0.7939
NGRBP02	0.0788	0.1001	100.1	0.7881	0.7873	
NGRBP03	0.0809	0.1002	100.2	0.8088	0.8071	
NGWBP01	0.0635	0.0999	99.9	0.6346	0.6352	0.6150
NGWBP02	0.0605	0.1002	100.2	0.6052	0.6040	
NGWBP03	0.0606	0.1000	100.0	0.6058	0.6058	
RHVP01	0.0650	0.0999	99.9	0.6504	0.6511	0.6640
RHVP02	0.0672	0.1002	100.2	0.6717	0.6704	
RHVP03	0.0671	0.1001	100.1	0.6711	0.6704	
NGGVP01	0.0748	0.0999	99.9	0.7485	0.7492	0.7852
NGGVP02	0.0851	0.1000	100.0	0.8511	0.8511	
NGGVP03	0.0754	0.0998	99.8	0.7538	0.7553	
RHBaliP01	0.0742	0.1001	100.1	0.7419	0.7412	0.7312
RHBaliP02	0.0780	0.0999	99.9	0.7798	0.7806	
RHBaliP03	0.0673	0.1002	100.2	0.6733	0.6719	
KOBaliP01	0.0643	0.1001	100.1	0.6430	0.6423	0.6396
KOBaliP02	0.0702	0.0998	99.8	0.7023	0.7037	
KOBaliP03	0.0573	0.1000	100.0	0.5727	0.5727	
NGRBaliP01	0.0696	0.0998	99.8	0.6963	0.6977	0.6455
NGRBaliP02	0.0576	0.1001	100.1	0.5758	0.5753	
NGRBaliP03	0.0664	0.1000	100.0	0.6636	0.6636	
NGGBaliP01	0.0534	0.1001	100.1	0.5336	0.5331	0.5811
NGGBaliP02	0.0555	0.1000	100.0	0.5550	0.5550	
NGGBaliP03	0.0657	0.1002	100.2	0.6566	0.6553	
NGWBaliP01	0.0409	0.1002	100.2	0.4094	0.4086	0.4201
NGWBaliP02	0.0454	0.1002	100.2	0.4538	0.4529	
NGWBaliP03	0.0400	0.1002	100.2	0.3998	0.3990	

APPENDIX B (CONT.)

Sample MG mg/g Kratom

The product's MG concentration in mg/g was calculated using the percentage to account for starting mass differences. This was done in the following equation:

$$(A / 100) \times 1000 \text{ mg} = B \text{ mg/g}$$

Where A is the percentage of MG calculated. Since A was a percentage and needed to be divided by 100 for use and because the result was multiplied by 1000 mg (1g), the equation was simplified to the following:

$$A \times 10 = B \text{ mg/g}$$

Using the Remarkable Herbs Vietnam Kratom from the example before, the equation becomes the following:

$$0.65109413 \times 10 = 6.5109413 \text{ mg/g}$$

Since all products were run in triplicate, this result was averaged when reported. This data can be seen in table XIII. Numbers have been rounded to 4 decimal places.

APPENDIX B (CONT.)

TABLE XIII

MITRAGYNINE CONTENT OF POWDER AND CAPSULE PRODUCTS

Name	Percentage of Product	Concentration (mg/g)	AVG Conc. (mg/g)	SD	%RSD
TKGSC01	0.3328	3.3285	3.5509	0.1845	5.1958
TKGSC02	0.3544	3.5440			
TKGSC03	0.3780	3.7802			
TKWSC01	0.3287	3.2870	3.3843	0.0928	2.7417
TKWSC02	0.3357	3.3567			
TKWSC03	0.3509	3.5092			
KKIC01	0.3340	3.3398	3.3114	0.0335	1.0127
KKIC02	0.3264	3.2643			
KKIC3	0.3330	3.3301			
NBBC01	0.3363	3.3627	3.3471	0.0144	0.4316
NBBC02	0.3328	3.3279			
NBBC03	0.3351	3.3508			
CKMaC01	0.3307	3.3069	3.3024	0.0094	0.2843
CKMaC02	0.3311	3.3109			
CKMaC03	0.3289	3.2893			
KORHP01	0.3263	3.2628	3.2695	0.0049	0.1506
KORHP02	0.3275	3.2745			
KORHP03	0.3271	3.2711			
KOTP01	0.3265	3.2649	3.2624	0.0024	0.0745
KOTP02	0.3263	3.2632			
KOTP03	0.3259	3.2591			
RHIP01	0.8856	8.8560	8.8025	0.0814	0.9248
RHIP02	0.8687	8.6875			
RHIP03	0.8864	8.8640			
RHTP01	1.1817	11.8171	10.4281	0.9870	9.4647
RHTP02	0.9853	9.8531			
RHTP03	0.9614	9.6141			

APPENDIX B (CONT.)

TABLE XIII (CONT.)

MITRAGYNINE CONTENT OF POWDER AND CAPSULE PRODUCTS

Name	Percentage of Product	Concentration (mg/g)	AVG Conc. (mg/g)	SD	%RSD
RHMaP01	0.9398	9.3978	9.0701	0.3144	3.4665
RHMaP02	0.8646	8.6460			
RHMaP03	0.9166	9.1664			
NGRSP01	0.6487	6.4872	7.0667	0.4640	6.5657
NGRSP02	0.7623	7.6230			
NGRSP03	0.7090	7.0898			
NGGSP01	0.9608	9.6076	10.5970	0.6996	6.6018
NGGSP02	1.1094	11.0936			
NGGSP03	1.1090	11.0898			
NBGMP01	0.8101	8.1010	8.6576	0.4038	4.6640
NBGMP02	0.8826	8.8256			
NBGMP03	0.9046	9.0463			
RHMP01	0.8836	8.8358	9.7684	0.7229	7.4000
RHMP02	0.9872	9.8720			
RHMP03	1.0597	10.5973			
OSMSP01	0.8014	8.0144	8.8048	0.6793	7.7155
OSMSP02	0.9673	9.6730			
OSMSP03	0.8727	8.7270			
NGRGP01	1.3976	13.9758	12.5607	1.2938	10.3005
NGRGP02	1.0849	10.8486			
NGRGP03	1.2858	12.8576			
NGWGP01	0.7229	7.2290	7.1393	0.0660	0.9241
NGWGP02	0.7072	7.0723			
NGWGP03	0.7117	7.1165			
NBRBP01	0.5956	5.9562	6.0527	0.1295	2.1392
NBRBP02	0.5966	5.9662			
NBRBP03	0.6236	6.2358			

APPENDIX B (CONT.)

TABLE XIII (CONT.)

MITRAGYNINE CONTENT OF POWDER AND CAPSULE PRODUCTS

Name	Percentage of Product	Concentration (mg/g)	AVG Conc. (mg/g)	SD	%RSD
NGRBP01	0.7872	7.8718	7.9388	0.0939	1.1822
NGRBP02	0.7873	7.8731			
NGRBP03	0.8071	8.0715			
NGWBP01	0.6352	6.3522	6.1503	0.1430	2.3244
NGWBP02	0.6040	6.0402			
NGWBP03	0.6058	6.0585			
RHVP01	0.6511	6.5109	6.6397	0.0910	1.3709
RHVP02	0.6704	6.7040			
RHVP03	0.6704	6.7040			
NGGVP01	0.7492	7.4923	7.8520	0.4663	5.9390
NGGVP02	0.8511	8.5105			
NGGVP03	0.7553	7.5532			
RHBaliP01	0.7412	7.4119	7.3123	0.4492	6.1432
RHBaliP02	0.7806	7.8058			
RHBaliP03	0.6719	6.7191			
KOBaliP01	0.6423	6.4235	6.3956	0.5352	8.3677
KOBaliP02	0.7037	7.0367			
KOBaliP03	0.5727	5.7267			
NGRBaliP01	0.6977	6.9773	6.4553	0.5160	7.9930
NGRBaliP02	0.5753	5.7527			
NGRBaliP03	0.6636	6.6358			
NGGBaliP01	0.5331	5.3307	5.8110	0.5319	9.1531
NGGBaliP02	0.5550	5.5498			
NGGBaliP03	0.6553	6.5525			
NGWBaliP01	0.4086	4.0856	4.2014	0.2347	5.5856
NGWBaliP02	0.4529	4.5286			
NGWBaliP03	0.3990	3.9899			

APPENDIX B (CONT.)

Liquid Sample MG Concentration

To calculate the MG concentration of the liquid samples, the following equation was used:

A mg/mL x Z x 10 mL = C mg

Where is the MG content of the samples as calculated by the software in mg/mL, and Z was the dilution factor of the sample. This was multiplied by 10 mL to get the total MG content of the sample since the samples were prepared in 10 mL of methanol. The result was the amount of MG in mg that came from the 1 mL of product used. Using the Zen Liquid Kratom as an example:

0.128704626 mg/mL x 4 x 10 mL = 5.14818504 mg
Or 5.14818504 mg/mL

Since all products were run in triplicate, this result was averaged when reported.

Total Liquid MG Content

Total MG content of the liquid samples was calculated by the following equation:

A mg/mL x B mL = C mg total

Where A is the average MG content of the samples as calculated in the previous equation and B is the total amount of mL in the product. Using the Zen Liquid Kratom product, which was an 8 mL product as an example

5.392312 mg/mL x 8 mL = 43.138496 mg

All data for the liquid samples can be seen in table XIV. Decimals have been rounded to 4 places.

APPENDIX B (CONT.)

TABLE XIV

MITRAGYNINE CONTENT OF LIQUID PRODUCTS

Name	Calc. Conc. (mg/mL)	Dilution	Final Conc. (mg/mL)	Liquid mg/mL	AVG	SD	%RSD	Total Product Content
RKPRL01	0.15885	2	0.3177	3.1771	3.2490	0.0945	2.9113	16.25
RKPRL02	0.15935	2	0.3187	3.1871				
RKPRL03	0.16913	2	0.3383	3.3826				
LKSL01	0.0103	1	DETECTED	DETECTED	-	-	-	-
LKSL02	0.0090	1	DETECTED	DETECTED				
LKSL03	0.0092	1	DETECTED	DETECTED				
BSSL01	0.0102	1	DETECTED	DETECTED	-	-	-	-
BSSL02	0.0134	1	DETECTED	DETECTED				
BSSL3	0.0115	1	DETECTED	DETECTED				
ZLKL01	0.12870	4	0.5148	5.1481	5.3923	0.1792	3.3246	43.14
ZLKL02	0.13637	4	0.5455	5.4551				
ZLKL03	0.13934	4	0.5574	5.5736				
OPLKL01	0.18541	4	0.7417	7.4166	6.8684	0.4078	5.9377	54.95
OPLKL02	0.16873	4	0.6749	6.7493				
OPLKL03	0.16098	4	0.6439	6.4392				

DETECTED = Samples were below the LOQ, but above the LOD.

APPENDIX C

7-hyrdroxymitragynine Calculations

Calculations for 7OH-MG used the same equations as the MG calculations. The concentration of MG calculated from the software were replaced with the concentration of 7OH-MG calculated by the software.

Data for the percentage of powder and capsule products for 7OH-MG can be seen in table XV. Data for the 7OH-MG content of powder and capsule products can be seen in table XVI. Data for the 7OH-MG content of liquid products can be seen in table XVII. All data has been rounded to 4 decimal places.

APPENDIX C (CONT.)

TABLE XV

7-HYDROXYMITRAGYNINE PERCENTAGE OF POWDER AND CAPSULE PRODUCTS

Name	Calc. Conc. (mg/mL)	Mass Used (g)	Mass Used (mg)	Total Conc. (mg)	Percentage	AVG %
TKGSC01	0.0203	0.1001	100.1	0.2027	0.2025	
TKGSC02	0.0203	0.1	100	0.2027	0.2027	0.2025
TKGSC03	0.0203	0.1002	100.2	0.2028	0.2024	
TKWSC01	0.0203	0.1	100	0.2027	0.2027	
TKWSC02	0.0203	0.1001	100.1	0.2027	0.2025	0.2026
TKWSC03	0.0203	0.1	100	0.2027	0.2027	
KKIC01	0.0204	0.1	100	0.2037	0.2037	
KKIC02	0.0203	0.1001	100.1	0.2031	0.2029	0.2031
KKIC3	0.0203	0.1	100	0.2028	0.2028	
NBBC01	LOD	0.0999	99.9			
NBBC02	LOD	0.1	100			
NBBC03	LOD	0.1	100			
CKMaC01	LOD	0.1002	100.2			
CKMaC02	LOD	0.1001	100.1			
CKMaC03	LOD	0.1	100			
KORHP01	0.0203	0.1	100	0.2027	0.2027	
KORHP02	0.0203	0.1001	100.1	0.2027	0.2025	0.2025
KORHP03	0.0203	0.1002	100.2	0.2027	0.2023	
KOTP01	0.0203	0.0999	99.9	0.2027	0.2029	
KOTP02	0.0203	0.1	100	0.2027	0.2027	0.2027
KOTP03	0.0203	0.1	100	0.2027	0.2027	
RHIP01	LOD	0.1	100			
RHIP02	LOD	0.1001	100.1			
RHIP03	LOD	0.0999	99.9			
RHTP01	LOD	0.1002	100.2			
RHTP02	LOD	0.1001	100.1			
RHTP03	LOD	0.1001	100.1			

LOD = Sample was below the limit of detection of 0.01mg/mL.

APPENDIX C (CONT.)

TABLE XV (CONT.)

7-HYDROXYMITRAGYNINE PERCENTAGE OF POWDER AND CAPSULE PRODUCTS

Name	Calc. Conc. (mg/mL)	Mass Used (g)	Mass Used (mg)	Total Conc. (mg)	Percentage	AVG %
RHMaP01	LOD	0.0999	99.9	-	-	-
RHMaP02	LOD	0.1002	100.2	-	-	
RHMaP03	LOD	0.1002	100.2	-	-	
NGRSP01	LOD	0.1002	100.2	-	-	-
NGRSP02	LOD	0.0999	99.9	-	-	
NGRSP03	LOD	0.1	100	-	-	
NGGSP01	LOD	0.1001	100.1	-	-	-
NGGSP02	LOD	0.1001	100.1	-	-	
NGGSP03	LOD	0.1	100	-	-	
NBGMP01	LOD	0.1002	100.2	-	-	-
NBGMP02	LOD	0.1	100	-	-	
NBGMP03	LOD	0.1	100	-	-	
RHMP01	LOD	0.1002	100.2	-	-	-
RHMP02	LOD	0.1001	100.1	-	-	
RHMP03	LOD	0.1002	100.2	-	-	
OSMSP01	LOD	0.1002	100.2	-	-	-
OSMSP02	LOD	0.1	100	-	-	
OSMSP03	LOD	0.1001	100.1	-	-	
NGRGP01	LOD	0.1	100	-	-	-
NGRGP02	LOD	0.1001	100.1	-	-	
NGRGP03	LOD	0.1001	100.1	-	-	
NGWGP01	LOD	0.1001	100.1	-	-	-
NGWGP02	LOD	0.1	100	-	-	
NGWGP03	LOD	0.1002	100.2	-	-	
NBRBP01	LOD	0.0999	99.9	-	-	-
NBRBP02	LOD	0.1001	100.1	-	-	
NBRBP03	LOD	0.1	100	-	-	

LOD = Sample was below the limit of detection of 0.01mg/mL.

APPENDIX C (CONT.)

TABLE XV (CONT.)

7-HYDROXYMITRAGYNINE PERCENTAGE OF POWDER AND CAPSULE PRODUCTS

Name	Calc. Conc. (mg/mL)	Mass Used (g)	Mass Used (mg)	Total Conc. (mg)	Percentage	AVG %
NGRBP01	LOD	0.1002	100.2	-	-	-
NGRBP02	LOD	0.1001	100.1	-	-	
NGRBP03	LOD	0.1002	100.2	-	-	
NGWBP01	LOD	0.0999	99.9	-	-	-
NGWBP02	LOD	0.1002	100.2	-	-	
NGWBP03	LOD	0.1	100	-	-	
RHVP01	LOD	0.0999	99.9	-	-	-
RHVP02	LOD	0.1002	100.2	-	-	
RHVP03	LOD	0.1001	100.1	-	-	
NGGVP01	LOD	0.0999	99.9	-	-	-
NGGVP02	LOD	0.1	100	-	-	
NGGVP03	LOD	0.0998	99.8	-	-	
RHBaliP01	LOD	0.1001	100.1	-	-	-
RHBaliP02	0.0156	0.0999	99.9	0.1555	0.1557	
RHBaliP03	LOD	0.1002	100.2	-	-	
KOBaliP01	LOD	0.1001	100.1	-	-	-
KOBaliP02	LOD	0.0998	99.8	-	-	
KOBaliP03	LOD	0.1	100	-	-	
NGRBaliP01	LOD	0.0998	99.8	-	-	-
NGRBaliP02	LOD	0.1001	100.1	-	-	
NGRBaliP03	LOD	0.1	100	-	-	
NGGBaliP01	LOD	0.1001	100.1	-	-	-
NGGBaliP02	LOD	0.1	100	-	-	
NGGBaliP03	LOD	0.1002	100.2	-	-	
NGWBaliP01	0.0142	0.1002	100.2	0.1419	0.1416	-
NGWBaliP02	LOD	0.1002	100.2	-	-	
NGWBaliP03	LOD	0.1002	100.2	-	-	

LOD = Sample was below the limit of detection of 0.01mg/mL.

APPENDIX C (CONT.)

TABLE XVI

7-HYDROXYMITRAGYNINE CONTENT OF POWDER AND CAPSULE PRODUCTS

Name	Percentage of Product	Concentration (mg/g)	AVG Conc. (mg/g)	SD	%RSD
TKGSC01	0.2025	2.0249	2.0253	0.0016	0.0780
TKGSC02	0.2027	2.0274			
TKGSC03	0.2024	2.0236			
TKWSC01	0.2027	2.0268	2.0261	0.0010	0.0484
TKWSC02	0.2025	2.0247			
TKWSC03	0.2027	2.0268			
KKIC01	0.2037	2.0370	2.0313	0.0041	0.2005
KKIC02	0.2029	2.0286			
KKIC3	0.2028	2.0281			
NBBC01	-	-	LOD	-	-
NBBC02	-	-			
NBBC03	-	-			
CKMaC01	-	-	LOD	-	-
CKMaC02	-	-			
CKMaC03	-	-			
KORHP01	0.2027	2.0268	2.0248	0.0017	0.0817
KORHP02	0.2025	2.0248			
KORHP03	0.2023	2.0228			
KOTP01	0.2029	2.0288	2.0275	0.0009	0.0449
KOTP02	0.2027	2.0268			
KOTP03	0.2027	2.0269			
RHIP01	-	-	LOD	-	-
RHIP02	-	-			
RHIP03	-	-			
RHTP01	-	-	LOD	-	-
RHTP02	-	-			
RHTP03	-	-			

LOD = Sample was below the limit of detection of 0.01mg/mL.

APPENDIX C (CONT.)

TABLE XVI (CONT.)

7-HYDROXYMITRAGYNINE CONTENT OF POWDER AND CAPSULE PRODUCTS

Name	Percentage of Product	Concentration (mg/g)	AVG Conc. (mg/g)	SD	%RSD
RHMP01	-	-	LOD	-	-
RHMP02	-	-			
RHMP03	-	-			
NGRSP01	-	-	LOD	-	-
NGRSP02	-	-			
NGRSP03	-	-			
NGGSP01	-	-	LOD	-	-
NGGSP02	-	-			
NGGSP03	-	-			
NBGMP01	-	-	LOD	-	-
NBGMP02	-	-			
NBGMP03	-	-			
RHMP01	-	-	LOD	-	-
RHMP02	-	-			
RHMP03	-	-			
OSMSP01	-	-	LOD	-	-
OSMSP02	-	-			
OSMSP03	-	-			
NGRGP01	-	-	LOD	-	-
NGRGP02	-	-			
NGRGP03	-	-			
NGWGP01	-	-	LOD	-	-
NGWGP02	-	-			
NGWGP03	-	-			
NBRBP01	-	-	LOD	-	-
NBRBP02	-	-			
NBRBP03	-	-			

LOD = Sample was below the limit of detection of 0.01mg/mL.

APPENDIX C (CONT.)

TABLE XVI (CONT.)

7-HYDROXYMITRAGYNINE CONTENT OF POWDER AND CAPSULE PRODUCTS

Name	Percentage of Product	Concentration (mg/g)	AVG Conc. (mg/g)	SD	%RSD
NGRBP01	-	-	LOD	-	-
NGRBP02	-	-			
NGRBP03	-	-			
NGWBP01	-	-	LOD	-	-
NGWBP02	-	-			
NGWBP03	-	-			
RHVP01	-	-	LOD	-	-
RHVP02	-	-			
RHVP03	-	-			
NGGVP01	-	-	LOD	-	-
NGGVP02	-	-			
NGGVP03	-	-			
RHBaliP01	-	-	LOD	-	-
RHBaliP02	0.1557	1.5568			
RHBaliP03	-	-			
KOBaliP01	-	-	LOD	-	-
KOBaliP02	-	-			
KOBaliP03	-	-			
NGRBaliP01	-	-	LOD	-	-
NGRBaliP02	-	-			
NGRBaliP03	-	-			
NGGBaliP01	-	-	LOD	-	-
NGGBaliP02	-	-			
NGGBaliP03	-	-			
NGWBaliP01	0.1416	1.4159	LOD	-	-
NGWBaliP02	-	-			
NGWBaliP03	-	-			

LOD = Sample was below the limit of detection of 0.01mg/mL.

APPENDIX C (CONT.)

TABLE XVII

7-HYDROXYMITRAGYNINE CONTENT OF LIQUID PRODUCTS

Name	Calc. Conc. (mg/mL)	Dilution	Final Conc. (mg/mL)	Liquid mg/mL	AVG	SD	%RSD	Total Product Content
RKPRL01	0.0242	2	0.0484	0.4843	0.4674	0.0187	4.0100	2.3370
RKPRL02	0.0221	2	0.0441	0.4413				
RKPRL03	0.0238	2	0.0477	0.4766				
LKSL01	0.0115	1	DETECTED	-	-	-	-	-
LKSL02	0.0119	1	DETECTED	-				
LKSL03	0.0146	1	DETECTED	-				
BSSL01	0.0023	1	DETECTED	-	-	-	-	-
BSSL02	0.0014	1	DETECTED	-				
BSSL3	0.0028	1	DETECTED	-				
ZLKL01	0.0084	4	DETECTED	-	-	-	-	-
ZLKL02	0.0110	4	DETECTED	-				
ZLKL03	0.0119	4	DETECTED	-				
OPLKL01	0.0129	4	DETECTED	-	-	-	-	-
OPLKL02	0.0127	4	DETECTED	-				
OPLKL03	0.0106	4	DETECTED	-				

DETECTED = Samples were below the LOQ, but above the LOD.

APPENDIX D

Paynantheine Calculations

Calculations for PAY used the same equations as the MG calculations. The concentration of MG calculated from the software were replaced with the concentration of PAY calculated by the software.

All data for PAY powder and liquid samples can be seen in table XVII and XVIII. All tables have been rounded to 4 decimal places.

APPENDIX D (CONT.)

TABLE XVIII

PAYNANTHEINE PERCENTAGE OF POWDER AND CAPSULE PRODUCTS

Name	Calc. Conc. (mg/mL)	Mass Used (g)	Mass Used (mg)	Total Conc. (mg)	Percentage	AVG %
TKGSC01	0.0230	0.1001	100.1	0.2302	0.2300	0.1975
TKGSC02	0.0181	0.1	100	DETECTED	0.1810	
TKGSC03	0.0182	0.1002	100.2	DETECTED	0.1815	
TKWSC01	0.0199	0.1	100	DETECTED	DETECTED	0.1826
TKWSC02	0.0173	0.1001	100.1	DETECTED	DETECTED	
TKWSC03	0.0176	0.1	100	DETECTED	DETECTED	
KKIC01	0.0169	0.1	100	DETECTED	DETECTED	0.1672
KKIC02	0.0165	0.1001	100.1	DETECTED	DETECTED	
KKIC3	0.0168	0.1	100	DETECTED	DETECTED	
NBBC01	0.0170	0.0999	99.9	DETECTED	DETECTED	0.1690
NBBC02	0.0169	0.1	100	DETECTED	DETECTED	
NBBC03	0.0168	0.1	100	DETECTED	DETECTED	
CKMaC01	0.0167	0.1002	100.2	DETECTED	DETECTED	0.1666
CKMaC02	0.0167	0.1001	100.1	DETECTED	DETECTED	
CKMaC03	0.0166	0.1	100	DETECTED	DETECTED	
KORHP01	0.0163	0.1	100	DETECTED	DETECTED	0.1632
KORHP02	0.0163	0.1001	100.1	DETECTED	DETECTED	
KORHP03	0.0164	0.1002	100.2	DETECTED	DETECTED	
KOTP01	0.0163	0.0999	99.9	DETECTED	DETECTED	0.1627
KOTP02	0.0163	0.1	100	DETECTED	DETECTED	
KOTP03	0.0163	0.1	100	DETECTED	DETECTED	
RHIP01	0.0289	0.1	100	0.2893	0.2893	0.2790
RHIP02	0.0248	0.1001	100.1	0.2476	0.2473	
RHIP03	0.0300	0.0999	99.9	0.3000	0.3003	
RHTP01	0.0345	0.1002	100.2	0.3453	0.3447	0.3167
RHTP02	0.0323	0.1001	100.1	0.3231	0.3228	
RHTP03	0.0283	0.1001	100.1	0.2830	0.2827	

DETECTED = Samples were below the LOQ, but above the LOD.

APPENDIX D (CONT.)

TABLE XVIII (CONT.)

PAYNANTHEINE PERCENTAGE OF POWDER AND CAPSULE PRODUCTS

Name	Calc. Conc. (mg/mL)	Mass Used (g)	Mass Used (mg)	Total Conc. (mg)	Percentage	AVG %
RHMP01	0.0320	0.0999	99.9	0.3200	0.3203	0.3021
RHMP02	0.0299	0.1002	100.2	0.2992	0.2986	
RHMP03	0.0288	0.1002	100.2	0.2880	0.2875	
NGRSP01	0.0229	0.1002	100.2	0.2292	0.2288	0.2451
NGRSP02	0.0267	0.0999	99.9	0.2667	0.2669	
NGRSP03	0.0240	0.1	100	0.2397	0.2397	
NGGSP01	0.0362	0.1001	100.1	0.3618	0.3615	0.3509
NGGSP02	0.0347	0.1001	100.1	0.3473	0.3470	
NGGSP03	0.0344	0.1	100	0.3443	0.3443	
NBGMP01	0.0268	0.1002	100.2	0.2684	0.2679	0.2781
NBGMP02	0.0280	0.1	100	0.2804	0.2804	
NBGMP03	0.0286	0.1	100	0.2860	0.2860	
RHMP01	0.0312	0.1002	100.2	0.3118	0.3112	0.3379
RHMP02	0.0333	0.1001	100.1	0.3334	0.3331	
RHMP03	0.0370	0.1002	100.2	0.3703	0.3695	
OSMSP01	0.0314	0.1002	100.2	0.3138	0.3132	0.3273
OSMSP02	0.0345	0.1	100	0.3451	0.3451	
OSMSP03	0.0324	0.1001	100.1	0.3239	0.3236	
NGRGP01	0.0500	0.1	100	0.5004	0.5004	0.4417
NGRGP02	0.0374	0.1001	100.1	0.3741	0.3737	
NGRGP03	0.0452	0.1001	100.1	0.4516	0.4511	
NGWGP01	0.0237	0.1001	100.1	0.2367	0.2365	0.2383
NGWGP02	0.0236	0.1	100	0.2362	0.2362	
NGWGP03	0.0243	0.1002	100.2	0.2426	0.2421	
NBRBP01	0.0289	0.0999	99.9	0.2887	0.2890	0.3184
NBRBP02	0.0321	0.1001	100.1	0.3205	0.3202	
NBRBP03	0.0346	0.1	100	0.3460	0.3460	

APPENDIX D (CONT.)

TABLE XVIII (CONT.)

PAYNANTHEINE PERCENTAGE OF POWDER AND CAPSULE PRODUCTS

Name	Calc. Conc. (mg/mL)	Mass Used (g)	Mass Used (mg)	Total Conc. (mg)	Percentage	AVG %
NGRBP01	0.0567	0.1002	100.2	0.5673	0.5661	0.6045
NGRBP02	0.0644	0.1001	100.1	0.6444	0.6438	
NGRBP03	0.0605	0.1002	100.2	0.6048	0.6036	
NGWBP01	0.0382	0.0999	99.9	0.3816	0.3820	0.3687
NGWBP02	0.0359	0.1002	100.2	0.3594	0.3587	
NGWBP03	0.0365	0.1	100	0.3655	0.3655	
RHVP01	0.0410	0.0999	99.9	0.4097	0.4101	0.4190
RHVP02	0.0375	0.1002	100.2	0.3747	0.3739	
RHVP03	0.0474	0.1001	100.1	0.4736	0.4731	
NGGVP01	0.0473	0.0999	99.9	0.4734	0.4739	0.5366
NGGVP02	0.0610	0.1	100	0.6105	0.6105	
NGGVP03	0.0524	0.0998	99.8	0.5244	0.5255	
RHBaliP01	0.0723	0.1001	100.1	0.7226	0.7219	0.6769
RHBaliP02	0.0696	0.0999	99.9	0.6958	0.6965	
RHBaliP03	0.0614	0.1002	100.2	0.6135	0.6123	
KOBaliP01	0.0587	0.1001	100.1	0.5869	0.5863	0.5624
KOBaliP02	0.0594	0.0998	99.8	0.5935	0.5947	
KOBaliP03	0.0506	0.1	100	0.5061	0.5061	
NGRBaliP01	0.0634	0.0998	99.8	0.6343	0.6355	0.5982
NGRBaliP02	0.0546	0.1001	100.1	0.5459	0.5453	
NGRBaliP03	0.0614	0.1	100	0.6137	0.6137	
NGGBaliP01	0.0509	0.1001	100.1	0.5087	0.5082	0.5331
NGGBaliP02	0.0521	0.1	100	0.5214	0.5214	
NGGBaliP03	0.0571	0.1002	100.2	0.5710	0.5699	
NGWBaliP01	0.0381	0.1002	100.2	0.3808	0.3800	0.3873
NGWBaliP02	0.0418	0.1002	100.2	0.4181	0.4173	
NGWBaliP03	0.0365	0.1002	100.2	0.3653	0.3646	

APPENDIX D (CONT.)

TABLE XIX

PAYNANTHEINE CONTENT OF POWDER AND CAPSULE PRODUCTS

Name	Percentage of Product	Concentration (mg/g)	AVG Conc. (mg/g)	SD	%RSD
TKGSC01	0.2300	2.2996			
TKGSC02	LOD	LOD	-	-	-
TKGSC03	LOD	LOD			
TKWSC01	LOD	LOD			
TKWSC02	LOD	LOD	-	-	-
TKWSC03	LOD	LOD			
KKIC01	LOD	LOD			
KKIC02	LOD	LOD	-	-	-
KKIC3	LOD	LOD			
NBBC01	LOD	LOD			
NBBC02	LOD	LOD	-	-	-
NBBC03	LOD	LOD			
CKMaC01	LOD	LOD			
CKMaC02	LOD	LOD	-	-	-
CKMaC03	LOD	LOD			
KORHP01	LOD	LOD			
KORHP02	LOD	LOD	-	-	-
KORHP03	LOD	LOD			
KOTP01	LOD	LOD			
KOTP02	LOD	LOD	-	-	-
KOTP03	LOD	LOD			
RHIP01	0.2893	2.8928			
RHIP02	0.2473	2.4733	2.7898	0.2283	8.1840
RHIP03	0.3003	3.0033			
RHTP01	0.3447	3.4465			
RHTP02	0.3228	3.2279	3.1671	0.2566	8.1026
RHTP03	0.2827	2.8268			

LOD = Sample was below the limit of detection of 0.01mg/mL

APPENDIX D (CONT.)

TABLE XIX (CONT.)

PAYNANTHEINE CONTENT OF POWDER AND CAPSULE PRODUCTS

Name	Percentage of Product	Concentration (mg/g)	AVG Conc. (mg/g)	SD	%RSD
RHMP01	0.3203	3.2033	3.0214	0.1364	4.5160
RHMP02	0.2986	2.9864			
RHMP03	0.2875	2.8747			
NGRSP01	0.2288	2.2878	2.4515	0.1604	6.5411
NGRSP02	0.2669	2.6693			
NGRSP03	0.2397	2.3974			
NGGSP01	0.3615	3.6149	3.5093	0.0754	2.1484
NGGSP02	0.3470	3.4698			
NGGSP03	0.3443	3.4434			
NBGMP01	0.2679	2.6786	2.7809	0.0760	2.7317
NBGMP02	0.2804	2.8038			
NBGMP03	0.2860	2.8604			
RHMP01	0.3112	3.1119	3.3795	0.2407	7.1214
RHMP02	0.3331	3.3311			
RHMP03	0.3695	3.6955			
OSMSP01	0.3132	3.1317	3.2729	0.1330	4.0622
OSMSP02	0.3451	3.4510			
OSMSP03	0.3236	3.2360			
NGRGP01	0.5004	5.0039	4.4175	0.5213	11.8003
NGRGP02	0.3737	3.7374			
NGRGP03	0.4511	4.5112			
NGWGP01	0.2365	2.3650	2.3826	0.0272	1.1398
NGWGP02	0.2362	2.3618			
NGWGP03	0.2421	2.4210			
NBRBP01	0.2890	2.8903	3.1841	0.2329	7.3134
NBRBP02	0.3202	3.2023			
NBRBP03	0.3460	3.4598			

APPENDIX D (CONT.)

TABLE XIX (CONT.)

PAYNANTHEINE CONTENT OF POWDER AND CAPSULE PRODUCTS

Name	Percentage of Product	Concentration (mg/g)	AVG Conc. (mg/g)	SD	%RSD
NGRBP01	0.5661	5.6613	6.0448	0.3170	5.2438
NGRBP02	0.6438	6.4376			
NGRBP03	0.6036	6.0357			
NGWBP01	0.3820	3.8203	3.6874	0.0979	2.6562
NGWBP02	0.3587	3.5871			
NGWBP03	0.3655	3.6547			
RHVP01	0.4101	4.1010	4.1905	0.4098	9.7783
RHVP02	0.3739	3.7394			
RHVP03	0.4731	4.7310			
NGGVP01	0.4739	4.7392	5.3661	0.5629	10.4908
NGGVP02	0.6105	6.1045			
NGGVP03	0.5255	5.2545			
RHBaliP01	0.7219	7.2192	6.7692	0.4685	6.9205
RHBaliP02	0.6965	6.9654			
RHBaliP03	0.6123	6.1231			
KOBaliP01	0.5863	5.8633	5.6237	0.3994	7.1028
KOBaliP02	0.5947	5.9469			
KOBaliP03	0.5061	5.0609			
NGRBaliP01	0.6355	6.3554	5.9819	0.3843	6.4244
NGRBaliP02	0.5453	5.4532			
NGRBaliP03	0.6137	6.1372			
NGGBaliP01	0.5082	5.0815	5.3314	0.2654	4.9782
NGGBaliP02	0.5214	5.2138			
NGGBaliP03	0.5699	5.6989			
NGWBaliP01	0.3800	3.8000	3.8729	0.2212	5.7106
NGWBaliP02	0.4173	4.1728			
NGWBaliP03	0.3646	3.6460			

APPENDIX D (CONT.)

TABLE XX

PAYNANTHEINE CONTENT OF LIQUID PRODUCTS

Name	Calc. Conc. (mg/mL)	Dilution	Final Conc. (mg/mL)	Liquid mg/mL	AVG	SD	%RSD	Total Product Content
RKPRL01	0.0471	0.5	0.0942	0.9423	0.9119	0.0240	2.6275	4.5597
RKPRL02	0.0442	0.5	0.0884	0.8837				
RKPRL03	0.0455	0.5	0.0910	0.9098				
LKSL01	LOD	-	-	-	-	-	-	-
LKSL02	LOD	-	-	-				
LKSL03	LOD	-	-	-				
BSSL01	LOD	-	-	-	-	-	-	-
BSSL02	LOD	-	-	-				
BSSL3	LOD	-	-	-				
ZLKL01	0.0283	0.25	0.1131	1.1307	1.1960	0.0493	4.1201	9.5681
ZLKL02	0.0302	0.25	0.1208	1.2077				
ZLKL03	0.0312	0.25	0.1250	1.2497				
OPLKL01	0.0579	0.25	0.2315	2.3152	1.9596	0.2596	13.2474	15.6766
OPLKL02	0.0465	0.25	0.1861	1.8608				
OPLKL03	0.0426	0.25	0.1703	1.7027				

LOD = Sample was below the limit of detection of 0.01mg/mL.

APPENDIX E

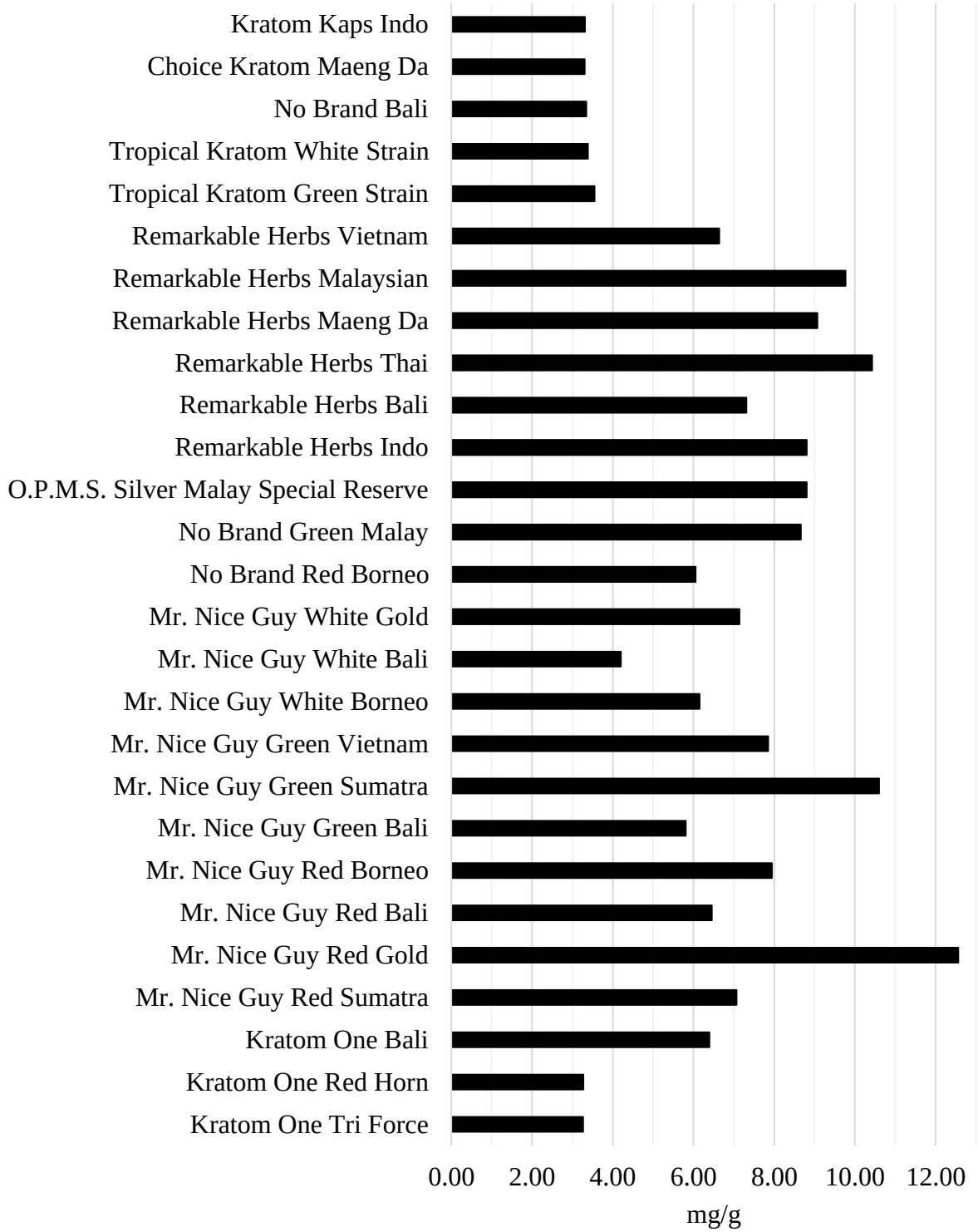

Figure 16. Mitragynine content of powder and capsule products

APPENDIX E (CONT.)

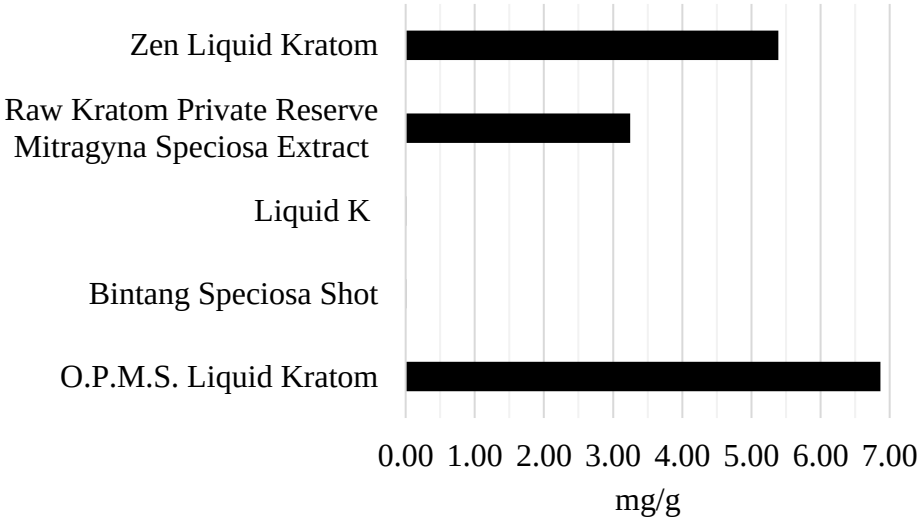

Figure 17. Mitragynine content of liquid products.

APPENDIX E (CONT.)

Figure 18. 7-hyrdroxymitragynine content of powder and capsule products.

APPENDIX E (CONT.)

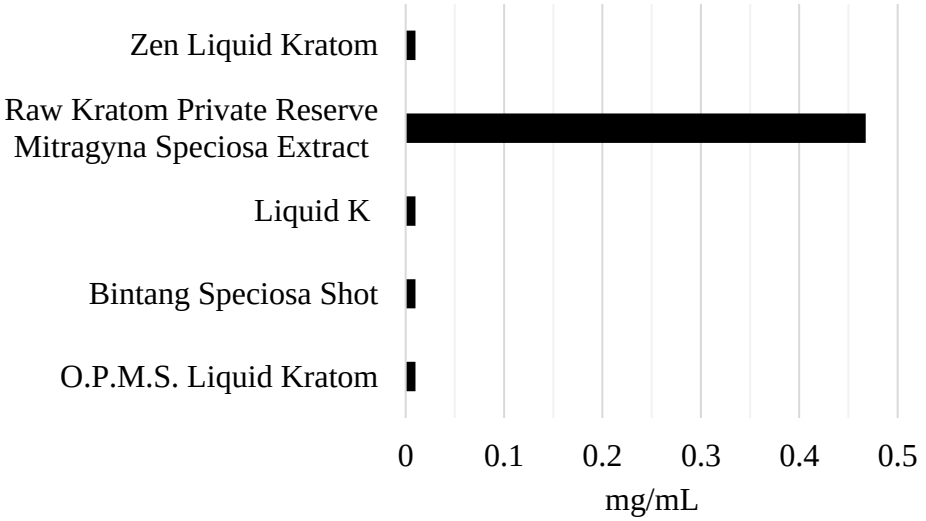

Figure 19. 7-hyrdroxymitragynine content of liquid products

APPENDIX E (CONT.)

Figure 20. Paynantheine content of powder and capsule products.

APPENDIX E (CONT.)

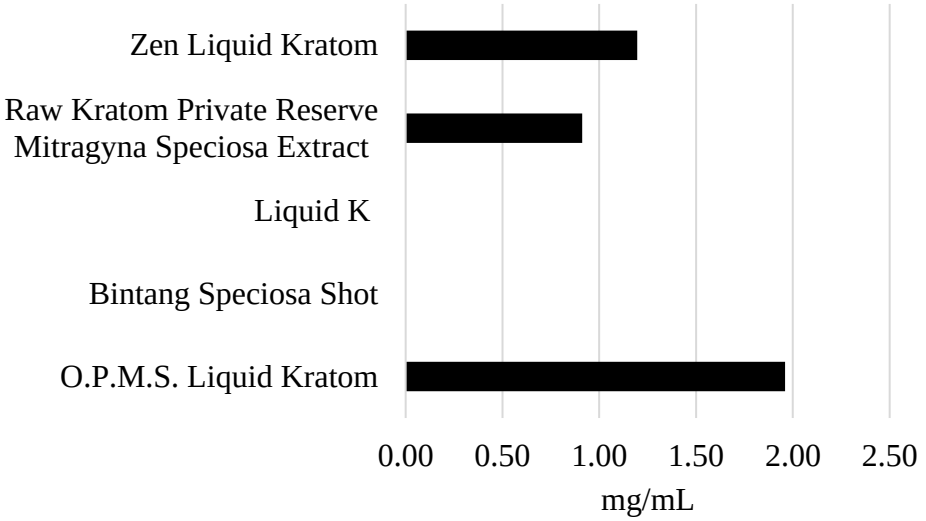

Figure 21. Paynantheine content of liquid products.

APPENDIX E (CONT.)

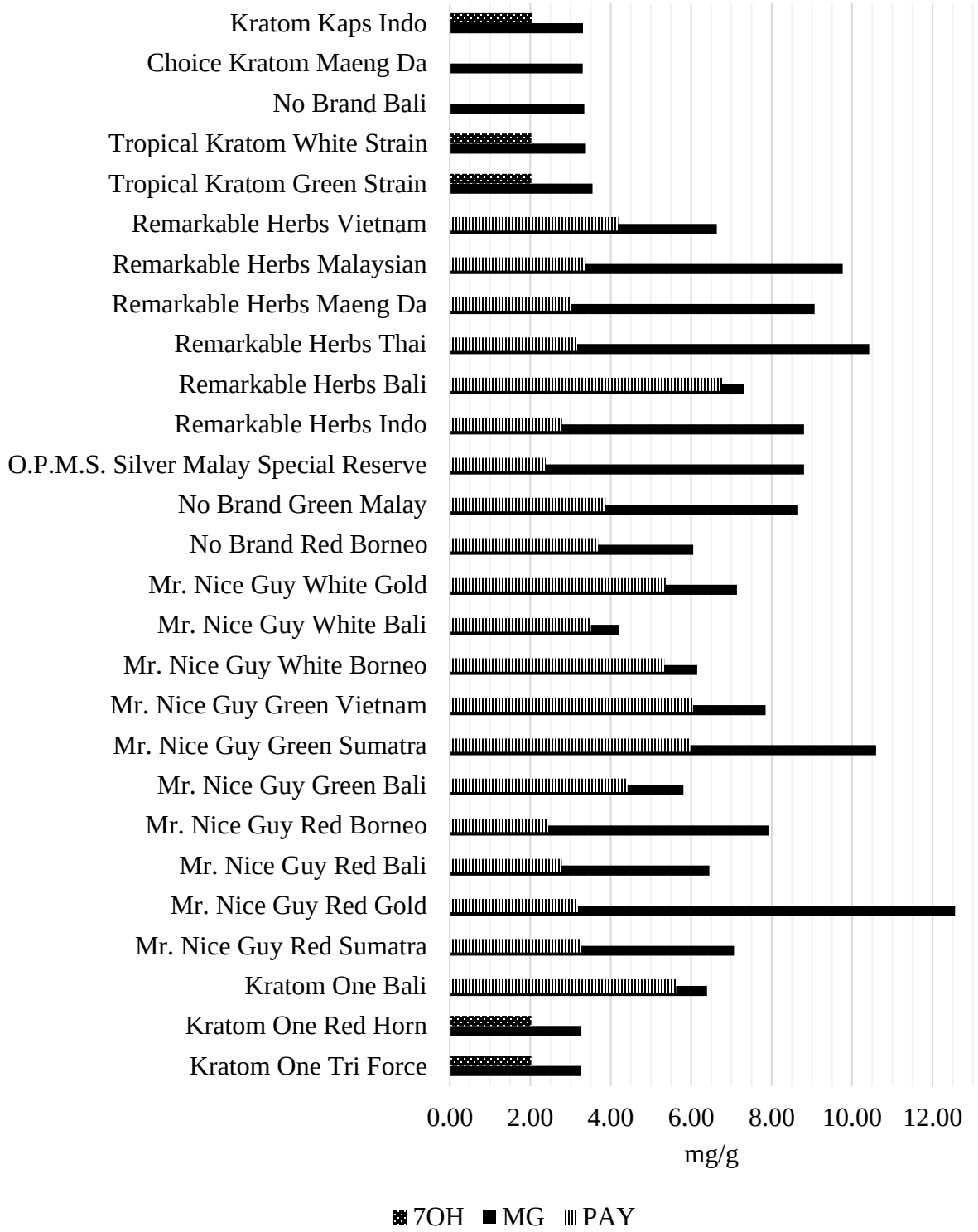

Figure 22. Mitragynie, 7-hydroxymitragynine, and paynantheine content of powder and capsule products.

APPENDIX E (CONT.)

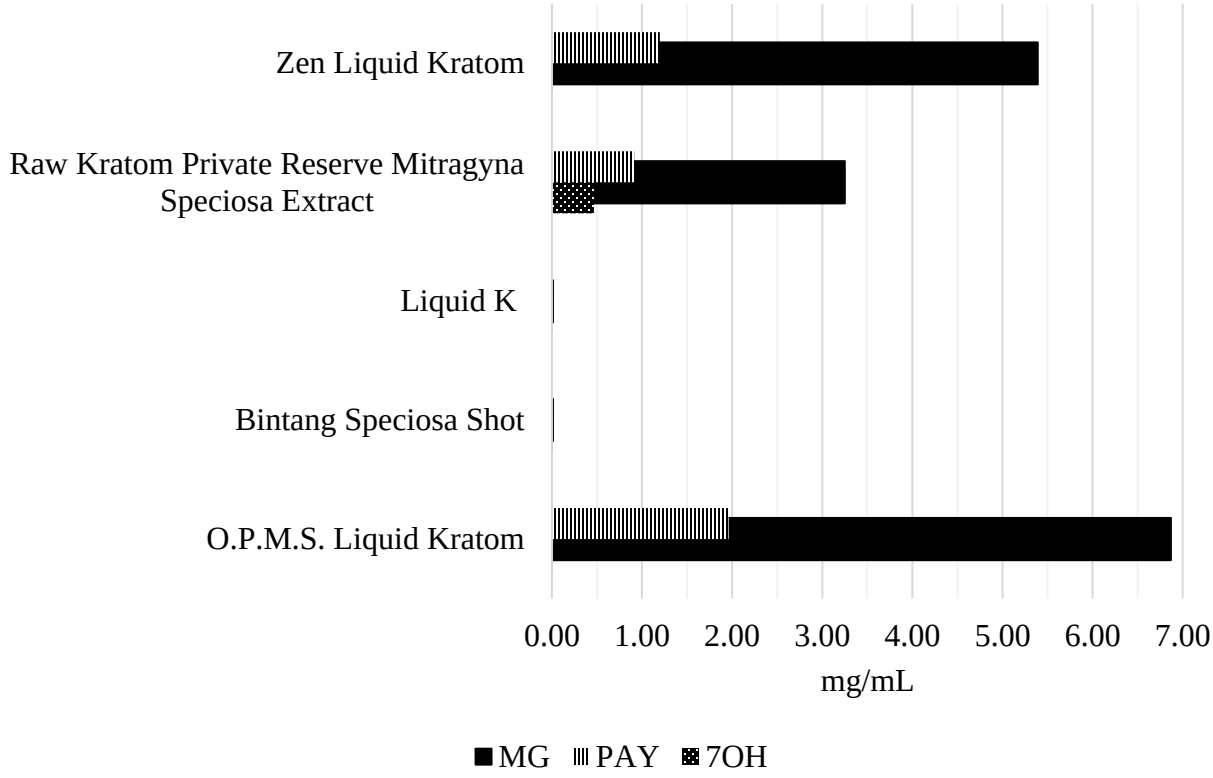

Figure 23. Mitragynie, 7-hydroxymitragynine, and paynantheine content of liquid products.

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ABSTRACTS: Wurzbach, J., Rybarczyk, K., McEntire, B.J., Shivers, B.L., Chancey, V.C. "Mass Properties Comparison of Dismounted and Ground Mounted Head-Supported Mass Configurations to Existing Performance and Acute Injury Risk Guidelines." Oral Presentation at the 2017 Military Health System Research Symposium (MHSRS), Kissimmee, FL.

Weisenbach, C.A., Bonts, T., Winegar, A., Rhodes, D., Daniel, R.W., Rybarczyk, K., Brozowski, F, Chancey, V.C. "A Method for Assessing Mandible Blunt Impact Biomechanics During Anterior-Posterior Impacts to Restrained Jaw." Poster Presentation at the 2017 American Society of Biomechanics (ASB) 41st Annual Meeting, Boulder, CO.

Wurzbach, J., Rybarczyk, K., McEntire, B.J., Shivers, B.L., Chancey, V.C. "Mass Properties Comparison of Dismounted and Ground Mounted Head-Supported Mass Configurations to Existing Performance and Acute Injury Risk Guidelines." Poster Presentation at the 2017 American Society of Biomechanics (ASB) 41st Annual Meeting, Boulder, CO.

Bonts, T., Rybarczyk, K., Sous, S., Brozowski, F. "Effects of Delamination Size and Depth on Impact Response and Tear Resistance of Composite Laminates Used in Aircrew Helmets." Oral Presentation at the 2017 Aerospace Medical Association Annual Scientific Meeting (AsMA), Denver, CO.

VITA (CONT.)

ABSTRACTS:

McEntire, B.J., Rybarczyk, K., Wursbach, J., Stieglitz, A., Chancey, V.C. "Toward Standardization: Headforms and Reference Coordinate Systems in Helmet Mass Properties Measurements." Oral Presentation at the 2016 SAFE Symposium 54th Annual Meeting, Dayton, OH.

Rybarczyk, K., Shivers, B.L., Dailey, J., Mathews, C., and Ranes, B. "Mixed Martial Arts Fighter Susceptibility to In-Fight Concussion: A Preliminary Analysis of Archival Data." Poster Presentation at the 2016 American College of Sport Medicine 63rd Annual Conference (ACSM), Boston, MA.

Rybarczyk, K., Shivers, B.L., Dailey, J., Mathews, C., and Ranes, B. "Mixed Martial Arts Fighter Susceptibility to In-Fight Concussion: A Preliminary Analysis of Archival Data." Oral Presentation at the 2016 American College of Sport Medicine 63rd Annual Conference (ACSM) Sports Interest Group, Boston, MA.

Rybarczyk, K., Shivers, B.L., Chancey, V.C., and McEntire, B.J. "Uniformity Assessment of Tri-Service Equipment and Methodology for Mass Properties Measurements of Protective Headgear." Oral Presentation at the 2016 Aerospace Medical Association Annual Scientific Meeting (AsMA), Atlantic City, NJ.

Brozoski, F., Rush, G.A., Weisenbach, C.A., Sous, S., and Rybarczyk, K. "Assessment of Maxillofacial Shield Protection During Blunt Impact Events." Oral Presentation at the 2015 Military Health System Research Symposium (MHSRS), Ft. Lauderdale, FL

Weisenbach, C.A., Logsdon, K., Salzar, R.S, Chancey, V.C., Brozoski, F. "Preliminary Investigation of Skull Fracture Patterns Using Impactor Representative of Helmet Back-Face Deformation". *Military Medicine*, Volume 183, Issue suppl_1, 1 March 2018, Pages 287–293, https://doi.org/10.1093/milmed/usx210