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The Analysis of Commercially Available Kratom Products in Richmond, Virginia

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Abstract

Kratom is a novel psychoactive substance that has gained popularity within the past ten years. Originating from Southeast Asia, the leaves of the *Mitragyna speciosa* tree contain two principal alkaloids, mitragynine and 7-hydroxymitragynine, that play a key role in opioid-like effects. Twenty-nine kratom products were obtained from tobacco shops in the Richmond, Virginia area, including powders, teas, capsules, extracts, and a carbonated beverage. Samples were analyzed using Direct Analysis in Real Time-Mass Spectrometry (DART-MS) for kratom alkaloids, labeled ingredients, and other possible organic compounds. Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) was used to quantitate aluminum, arsenic, copper, iron, magnesium, nickel, and lead with yttrium as the internal standard. Mitragynine and 7-hydroxymitragynine were present in every kratom sample. Kratom tea samples were found to have up to 20 times the tolerable upper intake of manganese. Overexposure to manganese can lead to Parkinsonian symptoms including tremors, dystonia, and facial muscle spasms. Gas Chromatography-Mass Spectrometry (GC-MS) was used to qualitatively confirm the presence of alkaloids and differentiate diastereomers. One non-kratom product was analyzed and was found to contain phenibut, an anxiolytic and nootropic substance. Phenibut was not listed on the label of this product. This work contributes to bring attention to the absence of quality control standards on kratom manufacturers as well as proper labeling of products sold at smoke and tobacco shops, prompting a public health concern due to the association of toxic metal levels in commercial kratom products.
Keywords: Kratom, Phenibut, Mitragynine, ICP-OES, DART-MS, GC-MS

Introduction

Background

Kratom is a novel psychoactive substance (NPS) that has gained popularity in the United States, Europe, and Australia in the past ten years[1]. Native to Southeast Asia, the leaves of the *Mitragyna speciosa* tree are ground into a powder for consumption. Originally, the leaves were chewed by manual laborers to aid in their vigorous, outdoor work. Today in the United States, the *M. speciosa* leaves are commonly brewed into a tea or capsulated for users to experience a "legal high". Prepared kratom teas are sold at Kava Bars, which provide a coffeehouse-like, casual atmosphere. Kratom powders can be purchased on the Internet, in smoke shops, and even in convenience stores.

More than 40 indole alkaloids are known to be present in the leaves of the *M. speciosa* tree[2]. Among them, mitragynine (66.2%), paynantheine (8.6%), speciogynine (6.6%), 7-hydroxymitragynine (2.0%), and speciociliatine (0.2%) are the most abundant (Figure 1)[3]. However, their content can vary according to the maturity of the plant. The percentage of mitragynine varies between younger and older plants, being much more abundant in older plants than the younger ones. The difference in the presence of the alkaloidal content in various species can be accounted for through environmental factors[4]. Moreover, with some alkaloids, the contents varied month to month[5]. Various researchers have found that alkaloid content in *Mitragyna* species can vary within individual plants depending on the provenance of the plant, part of the plant sampled, the age of the plant and/or the age of the plant tissue, as well as the time of year or season[5,6,7]. Of these alkaloids present, mitragynine and 7-hydroxymtiragynine are known to have psychoactive effects[3]. Speciociliatine and coryantheidine are also known to be

pharmacologically active[8]. Speciociliatine and speciogynine are stereoisomers of mitragynine, while paynantheine is a dehydro derivative of mitragynine[8]. 7-hydroxymitragynine has been found to be 17-fold and 30-fold more potent than morphine and mitragynine, respectively[9].

Uses & Effects

Different strains of kratom exist and can have different effects. Desired effects are dependent on the strain used as well as the dose. Low to moderate doses are defined as 1-5 g of kratom powder, which produce mild, stimulant-like effects[10]. Moderate to high doses are defined as 5-15 g of kratom powder, which produce opioid-like effects[10]. A dose greater than 15 g of kratom powder is considered a very high dose, which causes sedation and stupor[10].

Common strains of kratom include green vein, red vein, and white vein. The strain as well as potency are directly proportional to the amount of time that the *M. speciosa* leaf is grown[7]. The red strain is grown for the least amount of time and is the most potent[11]. The green strain is grown for a longer amount of time and is less potent[11]. The white strain is grown for an amount of time between the red and green strains. Additionally, the origin of the kratom is noted in the commercial name. For example, "Maeng Da" means pimp in Thai. Therefore, kratom obtained from young *M. speciosa* leaves in Thailand would be sold under the name "Red Vein Maeng Da".

Of these three popular strains, the red vein exhibits pain relieving effects[12].The white vein is a positive mood enhancer that increases alertness and concentration[12]. Some users consume white vein kratom in place of coffee. The green vein is described as between white and red veins, providing a mild energy enhancement[12]. The variety of effects in different strains

could be represented by the variation of the alkaloids in the plant in different moments of growing, showing a more mature plant with less opioid-like effects alkaloids. The variability of the presence of these alkaloids is not completely understood and deserves further investigation. The main hypothesis relies on the plant metabolism that could lead to the formation and/or transformation of alkaloids in the plant during the growing time of the plant. In addition to these desired effects, kratom is used to help relieve to withdrawal symptoms from other opioids[13]. Full effects of kratom are experienced 30-60 minutes after ingestion, lasting for 5-7 hours[14]. Specific conditions, such as prior food consumption, can impact the initial response[12]. To avoid legal issues, commercially sold kratom is labeled as "not for human consumption".

Kratom is a substance that is commonly used with other drugs. According to the Centers for Disease Control (CDC), fentanyl, fentanyl analogs, and heroin were the most common co-occurring substances in kratom overdose death cases[15]. Kratom was the only substance present in 7 of the 152 kratom-positive decedents[15]. From 2011 to 2017, the annual number of calls to United States poison centers increased 52.5-fold[16]. In 2017, an average of two calls per day regarded kratom exposure[16].

Further, various kratom concoctions exist that include combinations with other illicit substances. "4 x 100" is a cocktail that combines Coca-Cola, kratom leaves, and cough syrup, containing codeine, dextromethorphan, and diphenhydramine[17]. "4 x 100" has become popular in Thailand. The kratom cocktail has been fatal due to caffeine's enhancement of kratom alkaloid antinociceptive effects[18]. These effects are also enhanced by acetaminophen[17]. "Krypton" is a mixture of kratom leaves and O-desmethyltramadol[19]. "Krypton" was responsible for nine fatalities in Sweden within a one year period[20].

In 2016, a survey was conducted to gauge kratom usage in the United States. This survey was sent to 10,000 members of the American Kratom Association (AKA). Of the respondents (80.5%), the majority were male (56.91%), aged from 31-50 years old (55.09%), married or partnered (54.25%), made $35,000 or greater a year (63.24%), and had at least some college education (82.32%)[21]. According to the survey, decreased pain (85.01%), increased energy (83.75%), and less depressive mood (80.00%) were among the beneficial effects described for usage[21]. In order to achieve these beneficial effects, most users reported consuming 3 doses of up to 5 g per day, or 21 doses per week[21]. The exact number of kratom users in the United States is unknown, but experts suggest the number could exceed 1 million[22].

Pharmacology & Toxicology

Mitragynine is a basic and lipophilic drug, with a pK_a of 8.11 and a logP of 1.73[23], respectively. 7- hydroxymitragynine is also a basic drug and lipophilic, with a pK_a and logP of 12.20 and 2.2, respectively[24]. Due to their lipophilicity, mitragynine and 7-hydroxymitragynine are not soluble in water. The half-lives of mitragynine and 7-hydroxymitragynine are 3.5 h and 2.5 h, respectively[12]. The most common route of administration for kratom is oral[22], but smoking kratom has also been reported[25]. Both substances are excreted from the body through the urine[26].

While other kratom alkaloids, such as paynantheine and speciogynine, have not been found to be psychoactive, they may prove to be valuable biomarkers of kratom usage in the analysis of biological specimens due to their increased stability[27].

Toxic levels of mitragynine and 7-hydroxymitragynine in humans are poorly defined, with no toxic or lethal ranges. In case studies with kratom involved fatalities, postmortem blood concentrations of mitragynine ranged from 0.39-0.60 mg/L[26,28], 1.06 mg/L[9], and 0.02-0.18 μg/g[20] in whole blood. An *in vivo* rat model found the oral LD_{50} of mitragynine to be 547.7 mg/kg[29].

Mitragynine and 7-hydroxymitragynine both behave as partial agonists at the μ opioid receptor, competitive antagonists at the δ opioid receptor, and negligible effect at the κ receptor[30]. As previously mentioned, 7-hydroxymitragynine has a higher binding affinity for these receptors than that of mitragynine[10].

Kratom alkaloids are known to undergo both Phase I and Phase II metabolism[31]. Phase I metabolites of mitragynine include 7-hydroxymitragynine, 9-O-demethylmitragynine, 16-carboxymitragynine, and 9-O-demethyl-16-carboxymitragynine[32]. Metabolism of mitragynine is facilitated by cytochrome P450 (CYP450) isoforms, with major contributions from CYP34A and lesser contributions from CYP2D6 and CYP2C9[33]. Due to this, the usage of kratom with opioids that are also metabolically catalyzed by CYP34A, such as oxycodone, methadone, and fentanyl, may cause adverse drug-drug interactions[31]. Mitragynine has been determined to be a competitive inhibitor of CYP2D6, with IC_{50} and K_i values of 2.2 μM and 1.1 μM, respectively[34].

An *in vivo* blood-brain barrier (BBB) study discovered that less than 10% of unbound mitragynine and 7-hydroxymitragynine in plasma cross the BBB[35]. Yusof and collaborators also found that mitragynine and 7-hydroxymitragynine inhibit the efflux transporter P-glycoprotein (P-gp) in an *in vitro* model[35]. The EC_{50} values for P-gp inhibition were 18.2 μM and 32.4 μM for mitragynine and 7-hydroxymitragynine, respectively[24]. This indicates the possibility of drug-drug interactions with drugs that are P-gp substrates, such as loperamide, colchicine, and

morphine. Additionally, mitragynine was found to have a higher apical-to-basolateral permeability than 7-hydroxymitragynine[35]. β-arrestin binding is not elicited by mitragynine or 7-hydroxymitragynine[30].

Other adverse drug-drug interactions include the combination of kratom with Central Nervous System (CNS) depressants, which can lead to respiratory depression[36]. Adverse drug-drug interactions may also occur with other drugs that are metabolized by CYP34A, CYP2D6, and CYP2C9[37,38]. Doses of naltrexone that were used to antagonize morphine did not antagonize mitragynine in a rat model[39].

Recently, a metabolite of 7-hydroxymitragynine has been discovered in human plasma[40]. Mitragynine pseudoindoxyl is a result of a semipinacol rearrangement of 7-hydroxymitragynine[41]. It is known to be 31-fold more potent than 7-hydroxymitragynine and 119-fold more potent than mitragynine[42], with a 97.1% affinity for the μ opioid receptor[43].

Speciofoline, an oxindole alkaloid, is present in kratom at varying levels[44]. While an *in vitro* model found no affinity for the μ, δ, or κ receptors, two chemotypes were discovered[44]. These chemotypes are "low-speciofoline" and "high-speciofoline". Further, both chemotypes have similar inhibitory effects on CYP3A4, CYP2D6, and CYP2C9.

Legality & Classification

Though mitragynine and 7-hydroxymitragynine can cause opioid-like effects and are agonists at the μ opioid receptor, their classification as drugs remains ambiguous. Mitragynine and 7-hydroxymitragynine deviate from traditional opioids in the physiological, biochemical,

and behavioral effects that they produce[22]. Because of this, Raffa et al. have suggested a new class of drugs, known as "atypical opioids", that would include mitragynine and its analogs[3].

Presently, mitragynine and 7-hydroxymitragynine are not federally scheduled drugs in the United States. In 2016, the Drug Enforcement Agency (DEA) labeled kratom as a drug of concern and slated to classify it as a Schedule I drug[22]. However, kratom was never classified as a scheduled drug due to public demand, including petitions and public demonstrations. The possession, sale, and usage of kratom have been regulated at the state level. Alabama, Arkansas, Illinois, Indiana, Rhode Island, Wisconsin, and Vermont have banned kratom. In Wisconsin, for example, any amount of mitragynine present in the human body while operating a vehicle would result in a driving while intoxicated (DWI) charge. In 2018, the FDA released a report of 44 overdose deaths linked to kratom usage[45]. Kratom products were also linked to a *Salmonella* outbreak[46].

With the rise of kratom consumption, research surrounding its chemical profile and main active substances is of growing importance. The work outlined herein will assist in bridging the scientific community, kratom users, and policymakers when it comes to discuss the claimed benefits and the toxicity of kratom use

Relevance

Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) has been used to identify heavy metals in a variety of matrices, including drinking water[47], hair[48], and tea[49]. However, the author is not aware of any peer reviewed published reports using ICP-OES to evaluate the concentrations of heavy metals in kratom tea or other products. As the popularity of

kratom increases, it is important to have reliable and reproducible methods to detect heavy metals in kratom samples and/or products.

Experimental

Reagents & Standards

All reagents used were analytical grade or better. Solution preparations were made using ultrapure water (resistivity 18.2 MΩ/cm) obtained from a Direct-Q3 water purification system (Millipore Corporation, Burlington, MA, USA). Trace metal grade concentrated nitric acid (HNO_3) (67-70%) and concentrated hydrochloric acid (HCl) (34-37%) were obtained from Fisher Scientific (Waltham, MA, USA). The HNO_3 was diluted in ultrapure water to produce the 50% (v/v) washing solution and 2% (v/v) for dilution of standards and tea acidification. 30% hydrogen peroxide was obtained from Sigma Aldrich (St. Louis, MO, USA). VWR (Radnor, PA, USA) 0.45 μm syringe filters with cellulose acetate membranes were used to filter the samples before analysis by ICP-OES. For method development and validation, green tea leaves (*Camelia sinensis*) from NIST (SRM 3254) were used.

Polyelemental stock solution containing 100 ppm of silver, aluminum, arsenic, barium, boron, beryllium, calcium, cadmium, cerium, cobalt, chromium, cesium, copper, dysprosium, erbium, europium, gadolinium, gallium, holmium, iron, potassium, lanthanum, lutetium, magnesium, manganese, molybdenum, sodium, nickel, phosphorous, lead, sulfur, antimony, selenium, strontium, titanium, thorium, thallium, thulium, uranium, vanadium, ytterbium, and zinc (Inorganic Ventures, Christiansburg, VA, USA) was used to prepare the calibration curves.. A 100 ppm yttrium stock solution was purchased from Inorganic Ventures (Christiansburg, VA, USA). Yttrium was used as the internal standard at a concentration of 2 ppm in all standards, samples, and blanks Argon 99.999% pure (AirGas, Radnor, PA, USA) was used as auxiliary gas, to ignite the plasma, and to purge the optics.

Mitragynine and 7-hydroxymitragynine (100 µg/mL) analytical standards were purchased from Cerilliant (Round Rock, TX, USA). Paynantheine, corynoxine, speciogynine, and speciociliatine (1 mg) were purchased from Cayman Chemical (Ann Arbor, MI, USA). HPLC grade methanol was purchased from Fisher Scientific (Waltham, MA, USA) and used for dilutions and extractions.

Quantitation

In order to quantify trace metals in kratom samples, the internal standard method was used. Seven standards were prepared at concentrations of 0.01 mg/L 0.05 mg/L, 0.1 mg/L, 0.5 mg/L, 1 mg/L, 5 mg/L, and 10 mg/L in 2% HNO_3 at a final volume of 50 mL. Yttrium, the internal standard, was at a final concentration of 2 mg/L in each standard.

Sample Collection

A total of 29 kratom samples were purchased from 6 different local smoke shops in Richmond, Virginia. These samples included powders, capsules, teas, isolates/extracts, and a carbonated beverage. Different strains of kratom products were purchased, including red, white, green, Vietnam, Bali, and trainwreck, a blend of 11 different kratom strains. Of the 29 samples, 9 were powders, 2 were capsules, 12 were extracts/isolates, 5 were teas, and 1 was a carbonated beverage. All samples are listed by type in Tables 1-3, along with any information acquired from the label.

Sample Preparation

ICP-OES Analysis

Powder samples were subjected to an acid digestion. The acid digestion was modified from the Environmental Protection Agency (EPA) Method 3050B: Acid Digestion of Sediments, Soils, and Sludge. All glassware was washed with 50% HNO_3. 1 g of kratom powder was weighed and added to a clean 400 mL beaker along with 10% HNO_3. Samples were covered with watch glasses and heated over medium heat, without boiling, for 15 minutes. After heating, 5 mL of concentrated HNO_3 was added and samples were covered and heated for 30 minutes. This procedure was repeated three times until brown fumes dissipated. The samples were removed from heat, watch glasses were removed, and evaporated to 5 mL. Further, 2 mL of ultrapure water and 3 mL of cold 30% (v/v) H_2O_2 were added until effervescence subsided. 10 mL of concentrated hydrochloric acid (HCl) were added, covered, and heated for 15 minutes. Finally, the samples were filtered and transferred to a 50 mL volumetric flask, fortified with internal standard, and diluted with ultrapure water. The final solutions were stored at room temperature for analysis. A blank digestion was performed with no kratom sample. At the end of the acid digestion, the blank digestion was also fortified with internal standard at a concentration of 2 ppm.

Liquid samples, including extracts/isolates, teas, and the carbonated beverage, were acidified with 1 mL of concentrated HNO_3, filtered, and spiked with internal standard at a final concentration of 2 mg/L.

An Agilent 5110 inductively coupled plasma optical emission spectrometry (Santa Clara, CA, USA), with an Agilent SPS 4 autosampler was used for the simultaneous determination of

aluminum, arsenic, copper, iron, magnesium, manganese, nickel, lead, and yttrium in digested powder and acidified liquid samples. The ICP-OES was operated with a RF power of 1.40 kW, plasma flow rate of 12.0 L/min, auxiliary gas flow rate of 1.00 L/min, and nebulizer flow rate of 0.60 L/min. The plasma was positioned in radial viewing mode. The samples were introduced into the plasma via the nebulizer.

For ICP-OES analysis, multiple quality control measures were employed in order to validate the method. The method was validated in accordance with the EPA Method 6010D for ICP-OES. In addition to the internal standard, low, middle, and high controls were prepared at concentrations of 0.75 mg/L, 2.5 mg/L, and 7.5 mg/L, respectively, in order to assess interday and intraday bias. A standard reference material (NIST SRM 3254) was used to determine the accuracy of the method. Limit of detection (LOD) and limit of quantitation (LOQ) were also assessed by running a series of seven 2% HNO_3 blanks.

DART-MS Analysis

For Direct Analysis in Real Time-Mass Spectrometry (DART-MS), powder samples were subjected to an overnight methanolic extraction, as described by Fowble et al[50]. 1 g of kratom powder was weighed out and added to 10 mL of methanol. The solutions were agitated overnight and the supernatant was collected for DART analysis.

A JEOL JMS T100LC Accu-TOF DART-MS (JEOL USA, Inc, Peabody, MA, USA) was used in positive mode to analyze kratom samples. Orifice 1 was operated at 20 V while orifice 2 was operated at 5 V with a ring lens voltage of 3 V. Helium was used as the carrier gas with a heating temperature of 350ºC. The detector voltage was 2000 V and a mass range of 50 to 1500 m/z was used.

The instrument was calibrated with a methanolic solution of PEG 600. A positive control, containing methamphetamine, cocaine, and nefazodone assured that the exact masses fell within the appropriate range of ± 5 mmu. These standards were wanded first, followed by a blank. Each sample was wanded 7 times and a methanol blank was run between each sample to assess carryover.

GC-MS Analysis

For Gas Chromatography-Mass Spectrometry (GC-MS) analysis, 100 mg of powder samples were subjected to an overnight methanolic extraction. 100 mg of kratom powder was added to 1 mL of methanol. The solutions were agitated overnight and the supernatant was filtered and collected for GC-MS analysis. 10 µL of liquid samples, including extracts/isolates, teas, and the carbonated beverage were diluted in 1 mL of methanol for GC-MS analysis. A methanol blank was run alongside samples to ensure no carryover of analytes. Reference standards were used to positively identify kratom alkaloids by matching the retention time of the chromatography and respective mass spectrum.

A Shimadzu QP2020 GC-MS (Kyoto, Japan) was used in scan mode to analyze kratom samples. An Agilent HP-5MS column (30 m x 0.250 mm x 0.25 µm) was used along with helium as the carrier gas. The injection temperature was 250°C and spitless injection was used with an injection volume of 1 µL. The initial oven temperature was 100°C and ramped at 15°C/minute until 300°C with a final 6 minute hold. The total run time was 10.44 minutes.

Results & Discussion

ICP-OES Results

Calibration curves for each element were analyzed in triplicate and shown in Table 6. Linearity was achieved with all R^2 values exceeding 0.9980, as shown in Table 6. Equation 1 shows the calculation used to determine interday and intraday biases. The experimental value was obtained from the line of best fit of each calibration curve. For interday bias, the controls were analyzed on three separate days (n = 3). For intraday bias, the controls were analyzed on the same day (n = 3). All biases fell within ± 25%. Table 6 shows the percent difference between the calculated and known concentrations of analytes in the NIST SRM. All values fell within ± 22%. The LOD and LOQ for each element are shown in Table 6. Equation 2 and 3 show the calculations for LOD and LOQ, respectively. The standard deviation (σ)of six blank measurements was divided by the slope of the calibration curve, m.

$$Bias = \frac{Experimental - Actual}{Actual}\ x\ 100 \quad (1)$$

$$LOD = 3\ x\ \frac{\sigma}{m} \quad (2)$$

$$LOQ = 10\ x\ \frac{\sigma}{m} \quad (3)$$

Concentrations of trace elements in kratom samples are shown in Figures 3-8. Figures 9-18 show the consumption of trace elements in mg/serving based on suggested serving listed on the bottle. Equation 4 shows the calculation used to determine consumption of respective elements in mg/serving. The experimental value was obtained from the line of best fit of each calibration curve. This value was multiplied by the dilution factor and then the suggested serving

in L. Suggested servings for kratom teas, extracts/isolates, and carbonated beverage are shown in Table 8. For each sample, the suggested serving was the entire contents of the sample. None of the powder samples included suggested serving size on the label, thus the suggested serving was obtained by users' reports on the Internet and can vary from user to user. The average amount of kratom powder consumed by the users was defined as 8g/day. Uncertainty of the measurement was calculated by Equation 5. S_y represents the standard deviation of y, instrument response. The number of replicates is represented by k. The number of calibration points (7) is represented by n.

$$Consumption\ \left(\frac{mg}{Serving}\right) = Experimental\ \left(\frac{mg}{L}\right) x\ Dilution\ Factor\ x\ Suggested\ Serving\ \left(\frac{L}{Serving}\right) \quad (4)$$

$$Uncertainty\ in\ x\ (S_x) = \frac{s_y}{|m|}\sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y-\bar{y})^2}{m^2 \sum (x_i - \bar{x})^2}} \quad (5)$$

The data showed that the kratom samples analyzed in this study contained safe levels of aluminum, arsenic, copper, iron, magnesium, nickel, and lead. However, three kratom tea samples contained levels of manganese that exceed the tolerable upper intake level (UL) (Figure 13). The UL is the maximum amount of a substance that a person can consume on a daily basis with no adverse effects (Table 7). For adults, the UL of manganese is 11 mg/day[51]. The majority of cases reported to be associated to manganese toxicity are due to the occupational exposure of miners in manganese dioxide mines, or medical exposure as manganese is used as a contrast agent in medical diagnostics. However, manganese was also detected in a street drug called "Bazooka", a cocaine-based drug contaminated with manganese-carbonate from free base

preparation methods[52]. Cases of acute toxicity of manganese are rare, being the chronic toxicity associated to the accumulation of this metal in the organism[53].

Case studies of manganese toxicity include a male taking 4 mg of manganese daily for 2 weeks as part of a dietary supplement that reported insomnia, depression, delusions, and disorganized speech[54]. Three adults were accidentally given 60-80 g of manganese sulfate orally and experienced emesis, diarrhea, and elevated hepatic enzymes[55]. These cases display manganese toxicity at both the acute and chronic levels.

Overexposure of manganese can lead to what is known as "manganism". Manganism displays Parkinsonian symptoms, such as facial muscle spasms, dystonia, and trouble walking[56]. Manganism associated with kratom usage has not been reported in the literature. Manganese toxicity symptoms develop slowly, over the course of months and years[56]. Although the exact mechanism by which manganese induces neurotoxicity is poorly understood, several reports have suggested that manganese neurotoxicity may be associated with its interaction with other essential trace elements, including iron, zinc, copper and aluminum[53].

The three kratom tea samples with unsafe concentrations of manganese were purchased from the same tobacco shop and were produced by the same manufacturer. This highlights the need for stricter regulation of kratom products as well as adding manganese to the panel of metals tested for kratom quality assurance, if any is performed at all. In addition, the high levels of metals in kratom pose a danger to kratom users, specifically younger users as the brain is not fully developed until the age of 25.

The source of manganese in these samples is unknown. Manganese is a naturally occurring element and is found in soil at levels of 40-900 mg/kg[57]. In its natural form, manganese exists as manganese oxide. In acidic conditions, manganese oxide is reduced to

manganese[58]. In Southeast Asia, specifically Vietnam and Thailand, manganese levels in soil samples have been found to be elevated[58,59]. The higher levels of manganese in soil is due to the acidity of river water, which facilitates manganese depositing into soil sediments[59].

DART-MS Results

Mass spectra of kratom samples are shown in Figures 20-48. For kratom samples, principal alkaloids mitragynine and 7-hydroxymitragynine were found in every sample. However, mitragynine was not able to be differentiated between its diastereomers. Minor alkaloids were also present in the majority of samples. One sample, "Kratom Tea Lemon Flavor", was labeled as an "extract formula". In this sample, minor alkaloids were not found with DART (Figure 23).

In addition to kratom samples, one non-kratom sample was analyzed. The product was titled "Moon Water" and claimed to be for "energy and focus". Several compounds, such as pyrophosphate and sodium benzoate, were listed as ingredients and were found with DART.

GC-MS Results

The fragmentation of mitragynine and 7-hydroxymitragynine are shown in Figures 49 & 50, respectively. The GC-MS method was able to properly separate diastereomers of mitragynine, including speciogynine and speciociliatine. These peaks were confirmed by reference standards. A qualitative heat map is shown in Figure 19, which shows the confirmation of mitragynine and 7-hydroxymitragynine in every samples. The Kratom Tea Lemon Flavor was the only kratom sample that did not contain minor alkaloids.

Upon screening with GC-MS, the "Moon Water" sample was found to have phenibut (β-phenyl-γ-aminobutyric acid) present (Figure 2). This was confirmed by a reference standard (Figure 52). The mass spectrum for the suspected phenibut peak and the reference standard mass spectrum are shown in Figures 53 and 54, respectively. Phenibut is an anxiolytic and nootropic that was first utilized in Russia in the 1960's[60]. Phenibut is GABA-mimetic, primarily as a $GABA_B$ receptor agonist with minimal activity at the $GABA_A$ receptor[61]. The fragmentation pattern of phenibut is shown in Figure 51, with 104 *m/z* representing the loss of the phenyl group. This fragmentation results in the structure of GABA. The 161 *m/z* fragment is representative of cyclization into 4-phenyl-2-pyrrolidinone.

Phenibut is a drug that has been found in postmortem toxicology casework. In one case, phenibut was the only drug found[62]. However, the amount of phenibut was not quantitated. In one case from the North Carolina Office of Chief Medical Examiner, phenibut was found at a concentration of 64 mg/L in conjunction with 1,100 ng/mL of mitragynine, 67 mg/L of 7-aminoclonazepam, and 13 mg/L of pregabalin[63]. A total of 1,320 calls were made to US poison control centers from 2009-2019 with a sharp increase in calls since 2015[64].

Dependence has been reported in cases of daily phenibut consumption of 8 g for 10 months or greater[65,66]. Symptoms exhibited with overdose of phenibut include agitation, somnolence, hypothermia, delirium, hypotension, tachycardia, seizures, and coma[67,68,61,69].

Phenibut is readily available on the Internet and is not a controlled substance under the Controlled Substances Act in the United States. Adverse effects of phenibut include tremors, decreased appetite, agitation and insomnia[61].

The structure of phenibut is similar to baclofen (Figure 2), differing only by the presence of a chlorine atom in the para position of the phenyl ring. Baclofen has been used as a

withdrawal substance for phenibut dependence[70]. Phenibut withdrawal effects that have been reported include insomnia, anger, irritability, tremulousness, decreased appetite, and heart palpatations[71].

Conclusion

Herein, we provide a validated ICP-OES for the analysis of heavy metals in commercially available kratom products. Furthermore, we demonstrate that three kratom samples exhibited levels of manganese that are unsafe for consumption. Overexposure of manganese can lead to Parkinsonian symptoms, including dystonia, facial muscle spasms, and trouble walking.

The DART-MS method used was suitable for the analysis of kratom samples. The screening method was able to identify major alkaloids, minor alkaloids, and other ingredients listed or not on the label.

GC-MS was used to confirm the presence of alkaloids and other organic compounds. In one non-kratom sample, phenibut was present. Phenibut is a nootropic and anxiolytic compound that is an agonist at the $GABA_B$ receptor.

This work highlights the need for proper regulation within the kratom industry. From a public health perspective, it is important for users to be aware of the products that they are consuming. In addition, the elucidation of other organic compounds could be important for potential drug-drug interactions that are taking place. Future work with this project includes the validation of a GC-MS method in order to quantitate mitragynine and 7-hydroxymitragynine in kratom samples as well as phenibut in the Moon Water sample.

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Figures

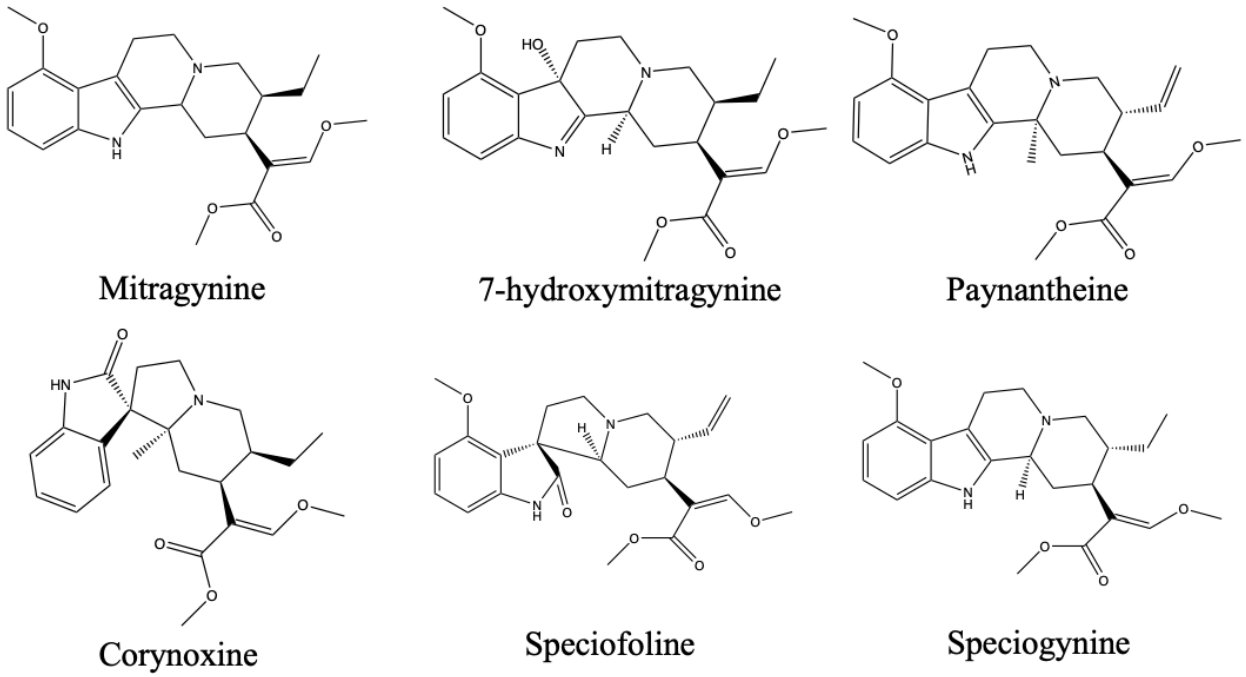

Figure 1: Structures of Kratom Alkaloids

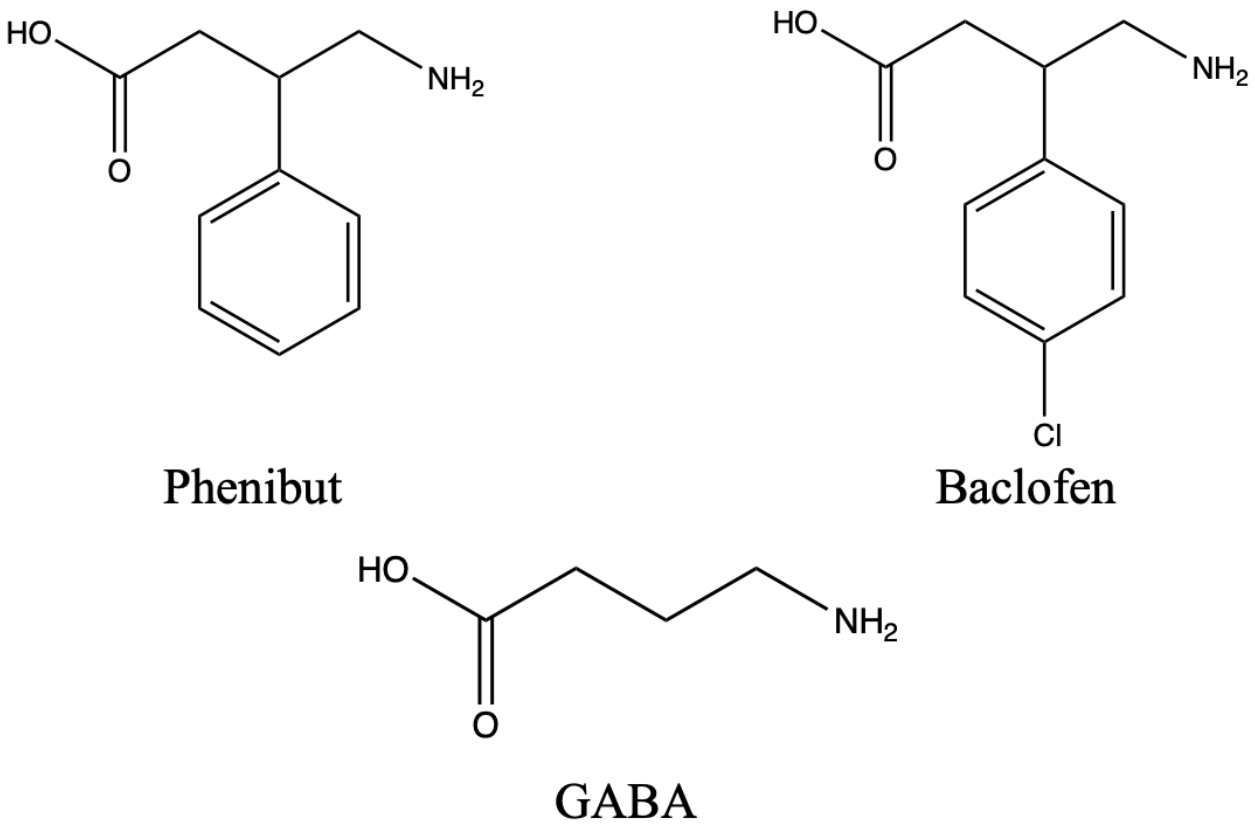

Figure 2: Structures of Phenibut, Baclofen, and GABA

Table 1: Kratom Powder Sample Information

Sample Name	Brand	Amount of Sample	Known Alkaloid Content	Other Ingredients	Lot Number
Mitragyna Speciosa White Thai	Natural Root	300 g	No Information Available	No Information Available	N/A
Mitragyna Speciosa Super Green	Natural Root	300 g	No Information Available	No Information Available	N/A
Mitragyna Speciosa White Meang Da	Natural Root	300 g	No Information Available	No Information Available	N/A
Mitragyna Speciosa Red Elephant	Natural Root	300 g	No Information Available	No Information Available	N/A
Trainwreck Kratom	Earth Kratom	250 g	No information available	No information Available	N/A
njoy Vietnam Powder	Njoy Supplements, LLC	200 g	1.1 g of Mitragyna speciosa, 1.6% mitragynine	No Information Available	N/A
njoy Bali Powder	Njoy Supplements, LLC	200 g	1.1 g of Mitragyna speciosa, 1.6% mitragynine	No Information Available	N/A
njoy Yellow Borneo Powder	Njoy Supplements, LLC	200 g	1.1 g of Mitragyna speciosa, 1.6% mitragynine	No Information Available	N/A
Kratom Tea Powder Supreme Blend	Zion Herbals	100 g	No Information Available	No Information Available	N/A

Table 2: Kratom Tea Sample Information

Sample Name	Brand	Manufacturer	Amount of Sample	Known Alkaloid Content	Other Ingredients	Lot Number
White Maeng Da Kratom Tea	Ultra Kratom Organics	Elite Distribution LLC	330 mL	8 g of kratom	Filtered Water, lemon juice from concentrate, lemon powder, citric acid, ascorbic acid, sugar	N/A
Red Maeng Da Kratom Tea	Ultra Kratom Organics	Elite Distribution LLC	330 mL	8 g of kratom	Filtered Water, lemon juice from concentrate, lemon powder, citric acid, ascorbic acid, sugar	N/A
Green Maeng Da Kratom Tea	Ultra Kratom Organics	Elite Distribution LLC	330 mL	8 g of kratom	Filtered Water, lemon juice from concentrate, lemon powder, citric acid, ascorbic acid, sugar	N/A
Kratom Tea Black Lemon Flavor	Label Kratom	APRU LLC	355 mL	Not Listed	Sparkling water, cane sugar, citric acid, black tea, carrot juice, natural flavor, glycerine, tartaric acid, proprietary blend	20345BLLT

Table 3: Kratom Extract Sample Information

Sample Name	Brand	Amount of Sample	Known Alkaloid Content	Other Ingredients	Lot Number
MIT 45 Gold Extract	MIT 45	15 mL	45% Mitragynine Extract	Not Listed	263
MIT 45 Gold Extract	MIT 45	15 mL	45% Mitragynine Extract	Not Listed	264
OPMS Liquid Kratom Extract	Optimized Plant Mediated Solutions (OPMS)	8.8 mL	280 mg of extract per bottle, 42% (118 mg/bottle) mitragynine, < 0.005% (< 0.01 mg) 7-hydroxymitragynine	None Listed	N/A
OPMS Liquid Kratom Extract	Optimized Plant Mediated Solutions (OPMS)	8.8 mL	280 mg of extract per bottle, 42% (118 mg/bottle) mitragynine, < 0.005% (< 0.01 mg) 7-hydroxymitragynine	None Listed	N/A
K Shot	Not Listed	15 mL	Not Listed	None Listed	N/A
K Shot	Not Listed	15 mL	Not Listed	None Listed	N/A
K80	Not Listed	10 mL	Not Listed	Filtered Water, Vegetable USP Kosher Glycerin, Ascorbic Acid, and Citric Acid	YB F21
Deja Vu	Deja Vu	10 mL	32 mg mitragynine per 5mL, 0 mg 7-hydroxymitragynine	Filtered Water, Tartaric acid, PEG400, sodium benzoate	170-20BW
OPMS Liquid Kratom Extract	Optimized Plant Mediated Solutions (OPMS)	8.8 mL	280 mg Mitragyna speciosa per bottle, 118 mg (42.0%) of mitragynine per bottle, <0.005% 7-hydroxymitragynine per bottle		221
24k Gold Kratom Extract	Zion Herbals	15 mL	32 mg of mitragynine per 5 mL, 0.02 mg of 7-hydroxymitragynine per 5 mL	Filtered water, lemon juice, PEG400, sodium benzoat	275-19BW
Lucky 80 Kratom Extract	Zion Herbals	15 mL	42 mg of mitragynine per 5 mL, 0 mg of 7-hydroxymitragynine	Filtered water, tartaric acid	045-20BW
Gold Reserve Kratom Extract	Zion Herbals	15 mL	Not Listed	filtered water, kosher vegetable glycerin	YB B1

Table 4: Intraday Bias

	Low (%)	Mid (%)	High (%)
Al	-9.4	12.8	4.6
As	11.1	2.5	-5.1
Cu	-0.1	0.9	-3.6
Fe	24.0	13.1	-0.2
Mg	21.2	14.7	0.3
Mn	-0.9	2.9	-3.0
Ni	2.6	1.3	-4.6
Pb	-20.2	-0.5	-6.7

(n = 9)

Table 5: Interday Bias

	Day 1			**Day 2**			**Day 3**		
	Low	Mid	High	Low	Mid	High	Low	Mid	High
Al	-9.4	12.8	4.6	2.5	1.8	2.1	7.6	6.1	10.6
As	11.1	2.5	-5.1	17.7	25.5	-7.2	-13.7	-16.0	14.4
Cu	-0.1	0.9	-3.6	18.3	2.7	1.6	17.3	11.4	14.2
Fe	24.0	13.1	-0.2	3.8	8.4	11.2	0.4	-1.4	23.2
Mg	21.2	14.7	0.3	3.7	11.6	3.6	7.2	21.6	14.5
Mn	-0.9	2.9	-3.0	7.6	9.2	10.6	5.0	3.8	9.2
Ni	2.6	1.3	-4.6	21.0	14.6	16.7	-6.3	-5.9	23.0
Pb	-20.2	-0.5	-6.7	7.5	16.3	21.5	-13.8	-18.4	14.8

(n = 9)

Table 6: Parameters of the Analytical Curves of Eight Heavy Metals (0.01-10 mg/L) Obtained by the Least Squares Method

Element	y = mx + b	Concentration Range (mg/L)	R^2	LOD (mg/L)	LOQ (mg/L)	SRM (%)
Aluminum	y = 0.01161x + 0.0033	0.01-10	0.9999	0.14	0.49	-8.8
Arsenic	y = 0.000699x + 0.00025	0.01-10	0.9999	0.21	0.70	-22.2
Copper	y = 0.011140x + 0.00110	0.01-10	0.9999	0.08	0.26	9.7
Iron	y = 0.01487x + 0.0015	0.01-10	0.9999	0.07	0.25	-9.5
Magnesium	y = 0.0523x + 0.007	0.01-10	0.9983	0.08	0.27	N/A
Manganese	y = 0.1169x + 0.002	0.01-10	0.9999	0.01	0.04	-4.5
Nickel	y = 0.00244x + 0.00025	0.01-10	0.9981	0.16	0.54	N/A
Lead	y = 0.0011191x + 0.000496	0.01-10	0.9999	0.32	1.0	14.4

Table 7: Tolerable Upper Intake Level of Heavy Metals Per Day

Metal	Tolerable Upper Intake Level (mg)
Al	120
Cu	10
Fe	45
Mg	350
Mn	11
Ni	1

Table 8: Suggested Serving of Kratom Tea & Extract Samples

Sample	Suggested Serving (mL)
White Maeng Da Kratom Tea	330
Red Maeng Da Kratom Tea	330
Green Maeng Da Kratom Tea	330
Kratom Tea Lemon Flavor	355
Moon Water	237
Speciosa Soda	355
MIT 45 Gold Extract 1	15
MIT 45 Gold Extract 2	15
OPMS Liquid Kratom Extract 1	8.8
OPMS Liquid Kratom Extract 2	8.8
K Shot 1	15
K Shot 2	15
K80	10
Deja Vu	10
OPMS Liquid Kratom Extract 3	8.8
24K Gold Kratom Extract	15
Lucky 80 Kratom Extract	15
Gold Reserve Kratom Extract	15

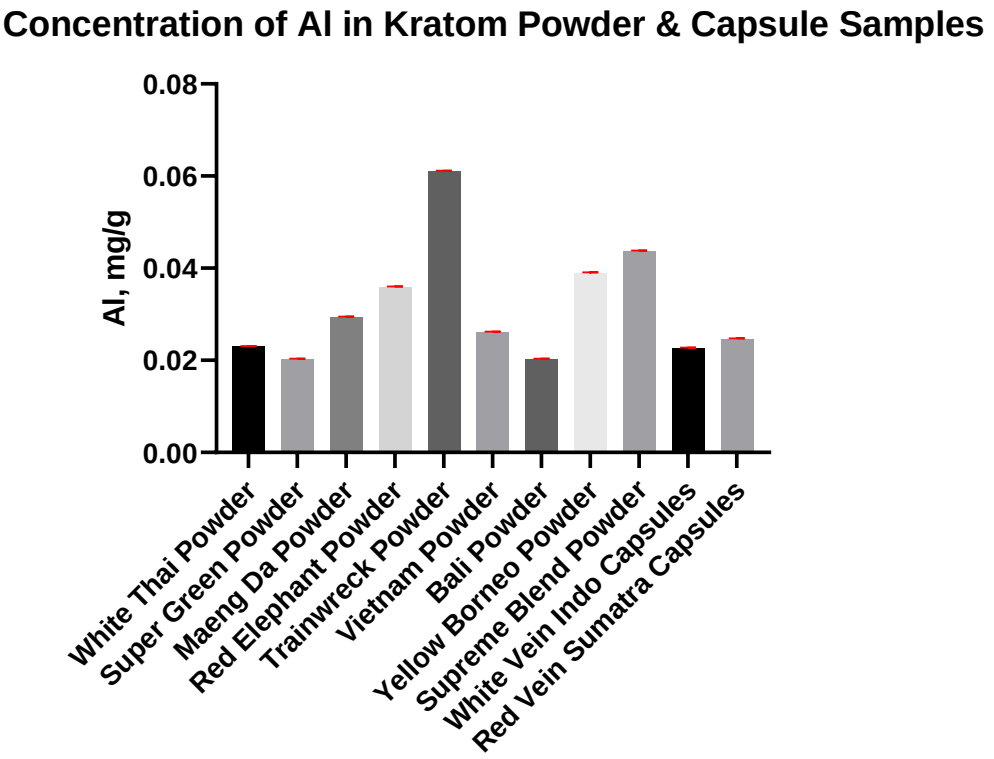

Figure 3: Concentration of Al in Kratom Powder & Capsule Samples

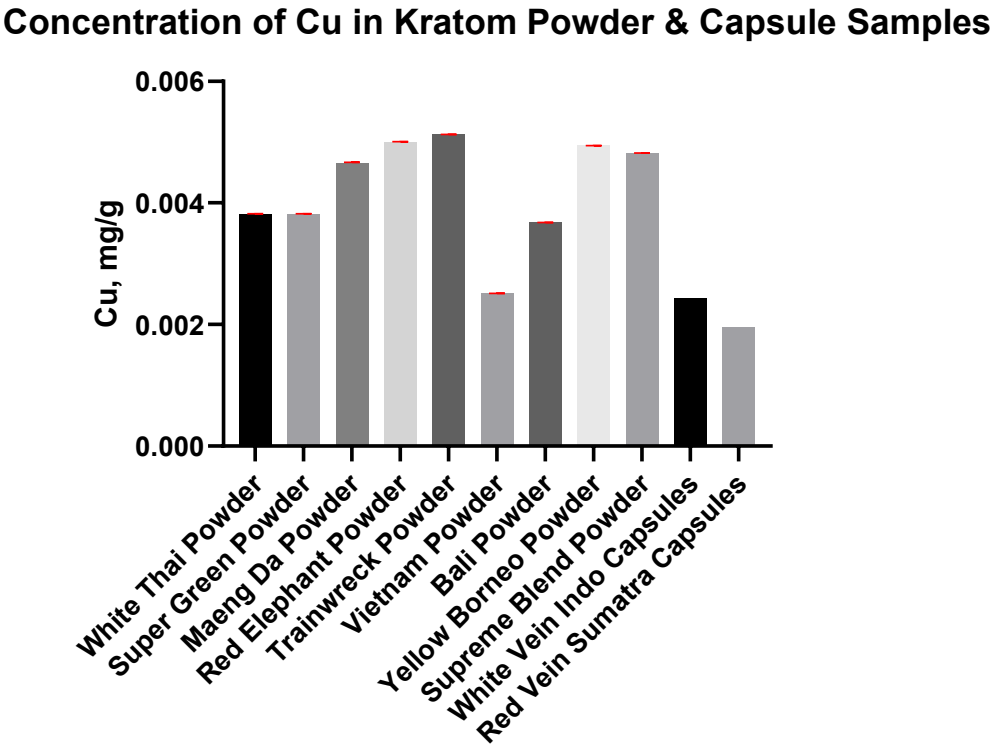

Figure 4: Concentration of Cu in Kratom Powder & Capsule Samples

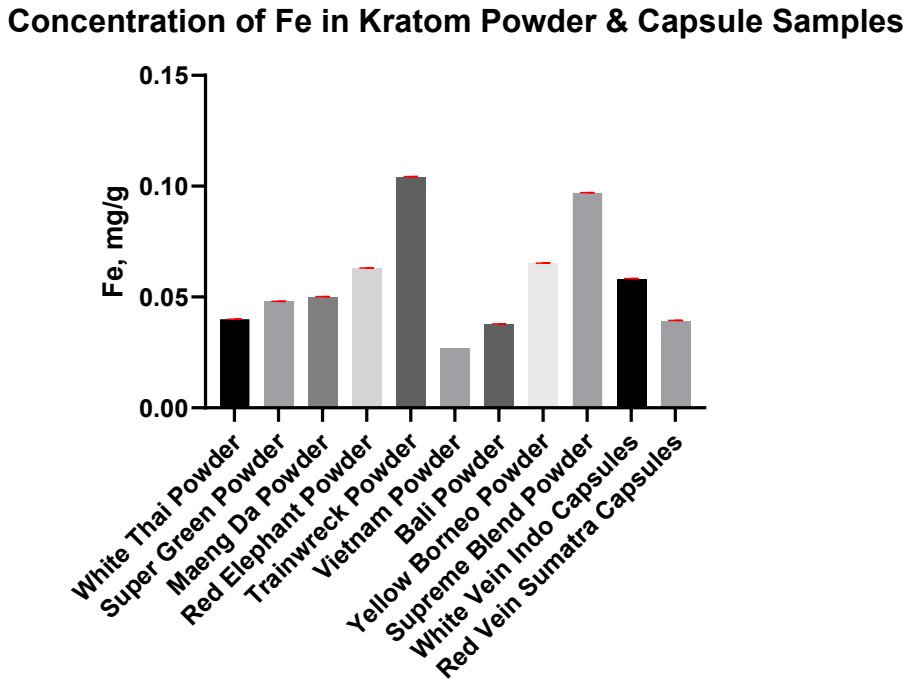

Figure 5: Concentration of Fe in Kratom Powder & Capsule Samples

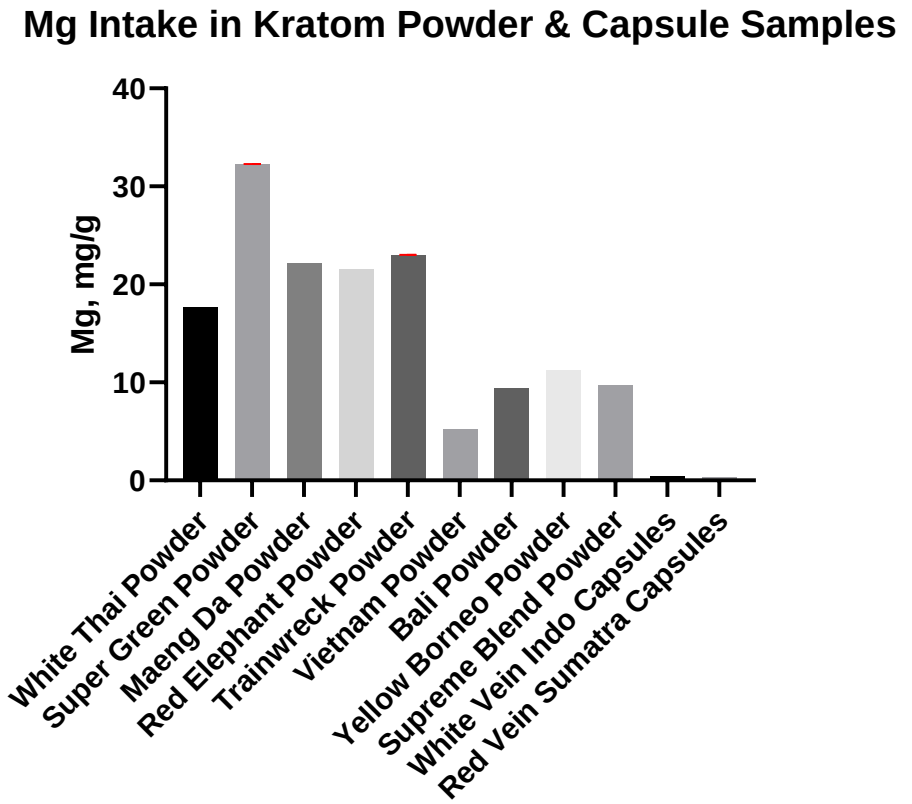

Figure 6: Concentration of Mg in Kratom Powder & Capsule Sample

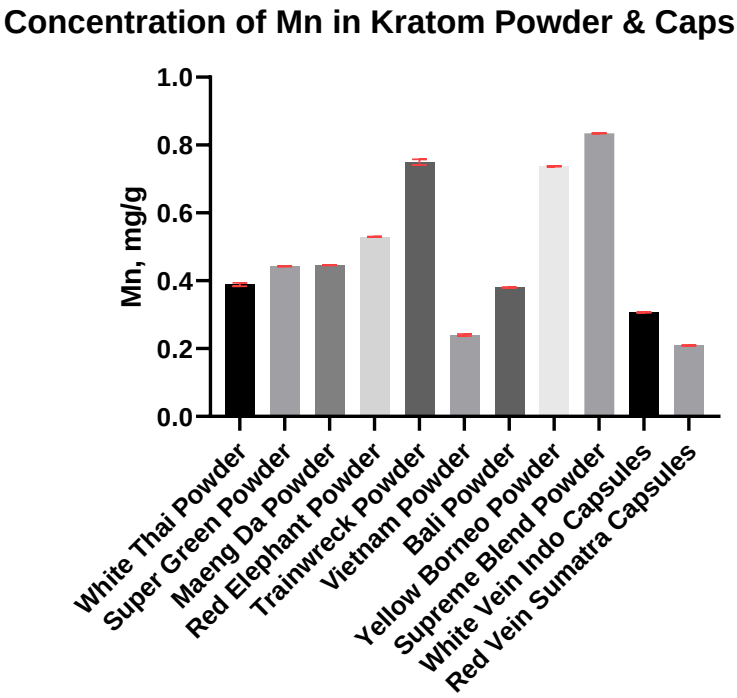

Figure 7: Concentration of Mn in Kratom Powder & Capsule Samples

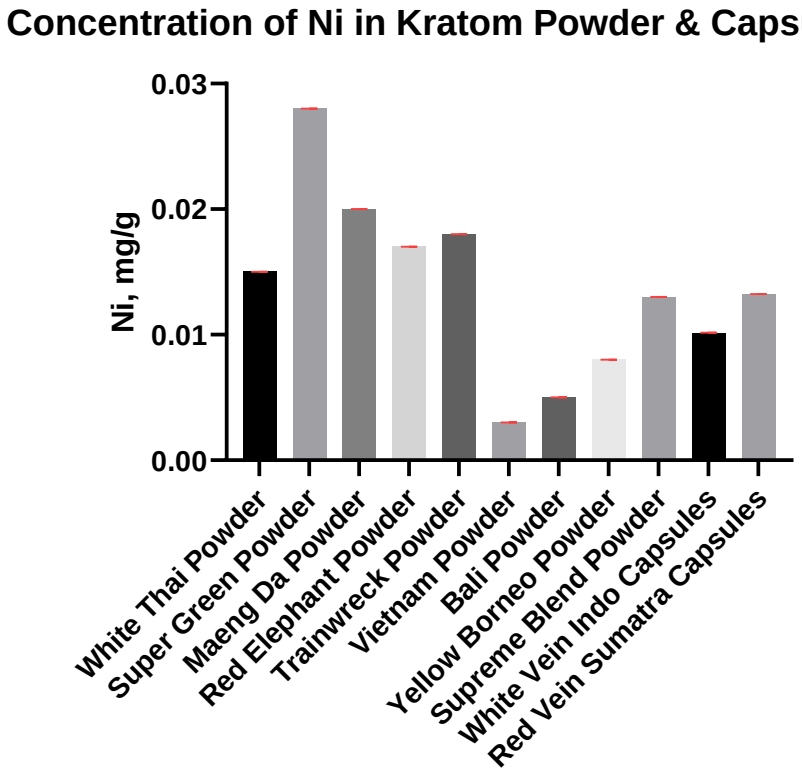

Figure 8: Concentration of Ni in Kratom Powder & Capsule Samples

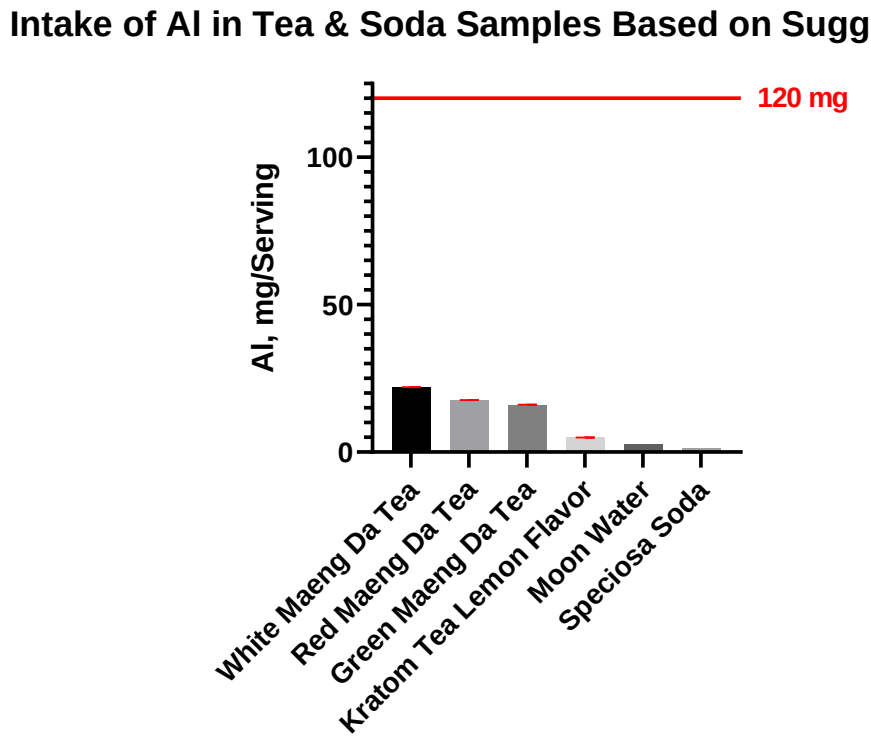

Figure 9: Intake of Al in Tea & Soda Samples Based on Suggested Serving

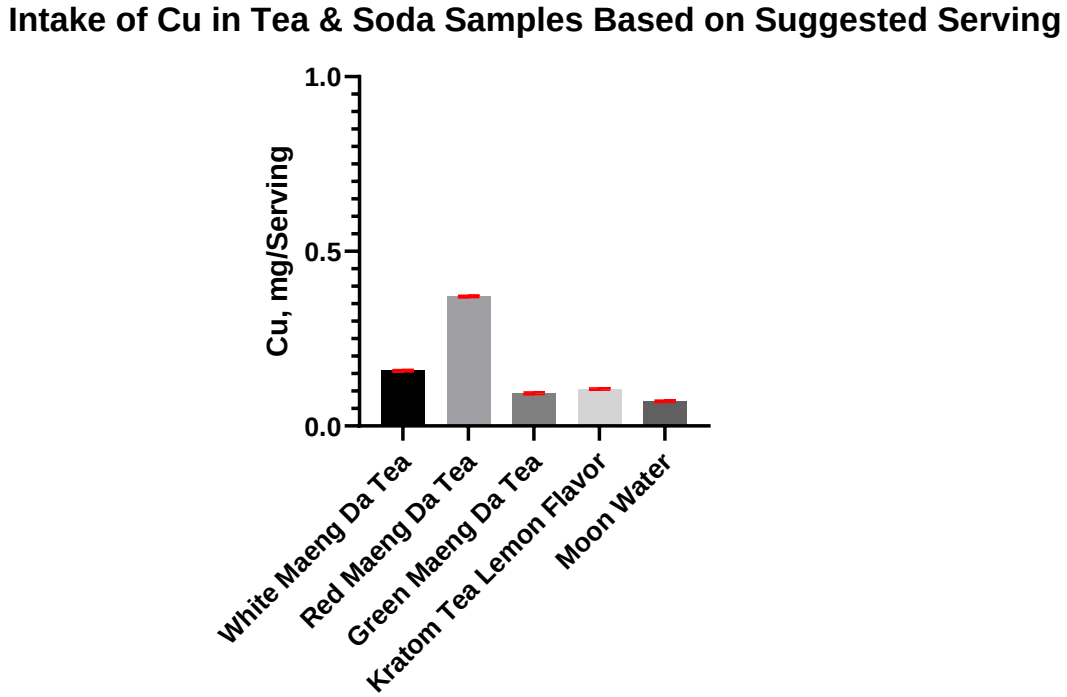

Figure 10: Intake of Cu in Tea & Soda Samples Based on Suggested Serving

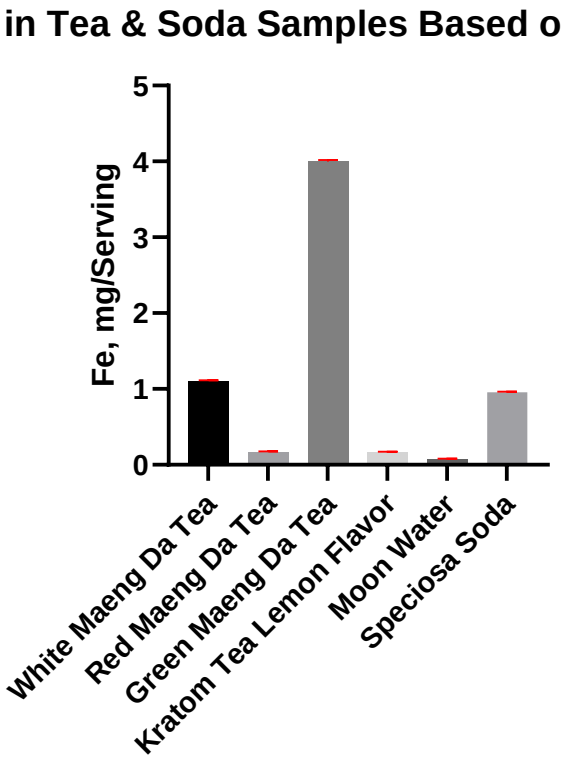

Figure 11: Intake of Fe in Tea & Soda Samples Based on Suggested Serving

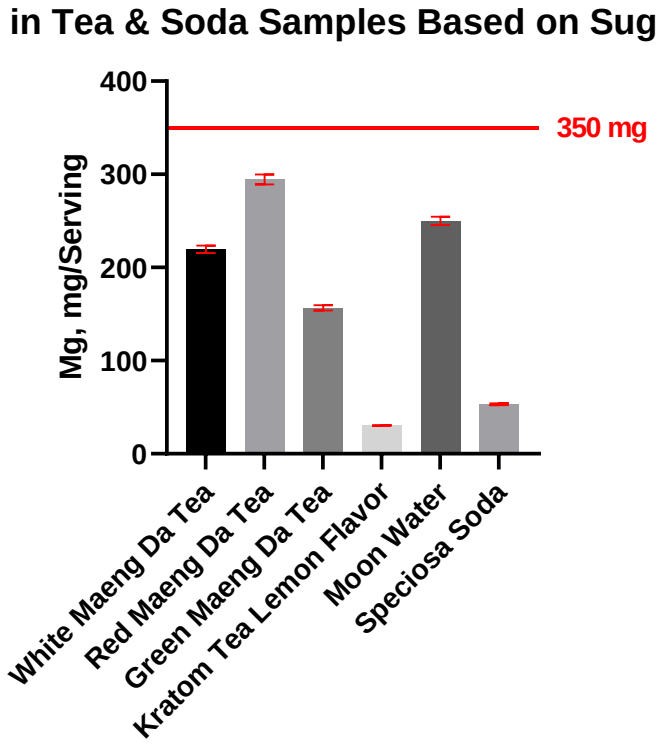

Figure 12: Intake of Mg in Tea & Soda Samples Based on Suggested Serving

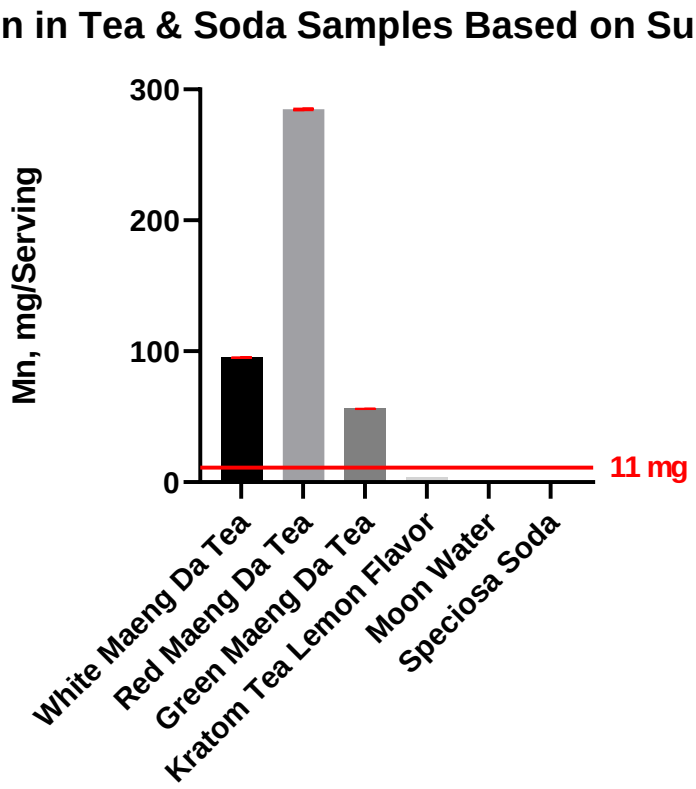

Figure 13: Intake of Mn in Tea & Soda Samples Based on Suggested Serving

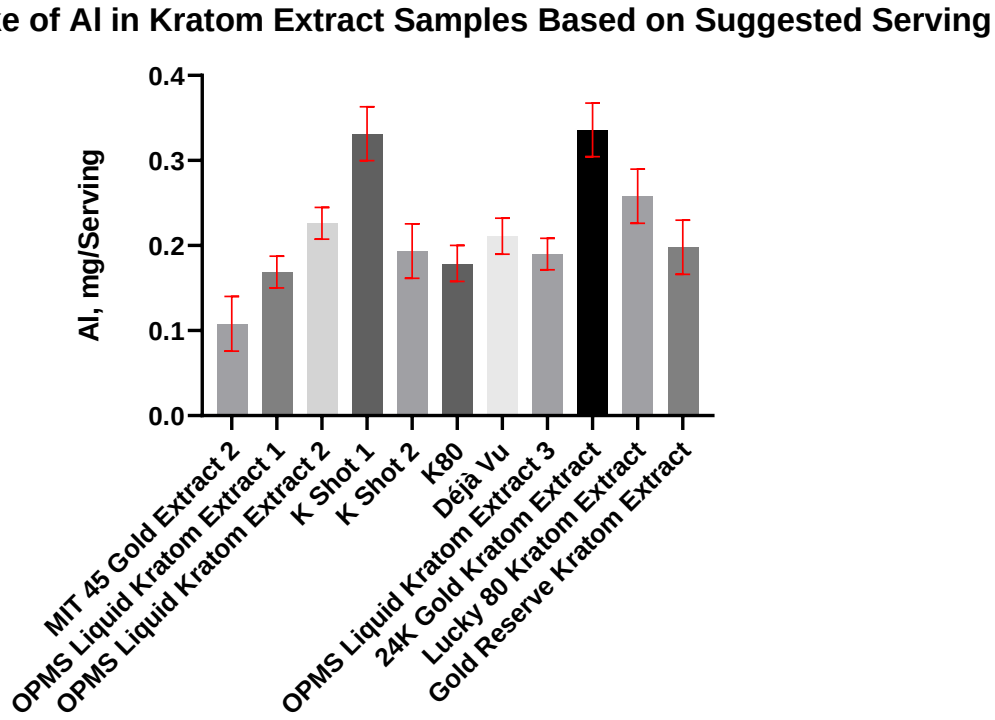

Figure 14: Intake of Al in Kratom Extract Samples Based on Suggested Serving

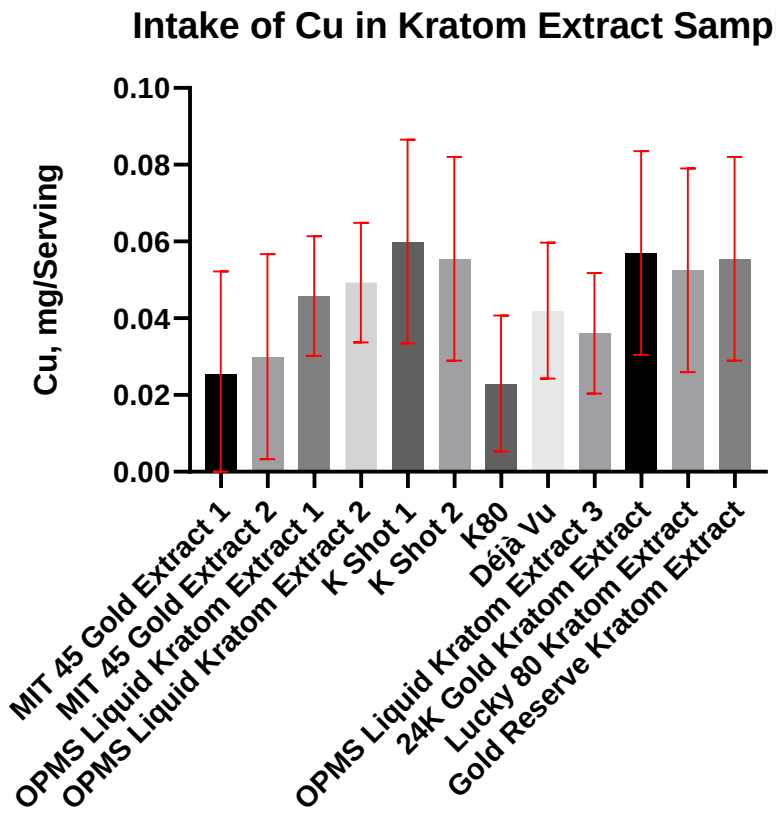

Figure 15: Intake of Cu in Kratom Extract Samples Based on Suggested Serving

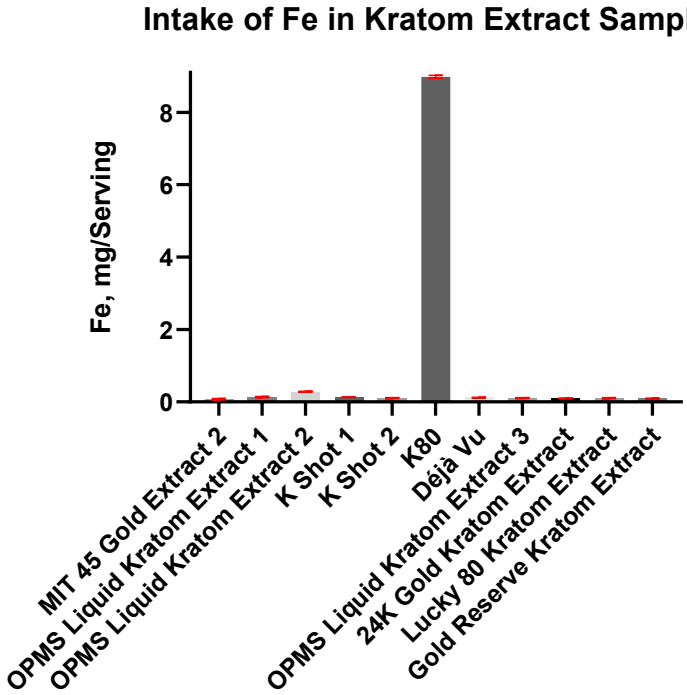

Figure 16: Intake of Fe in Kratom Extract Samples

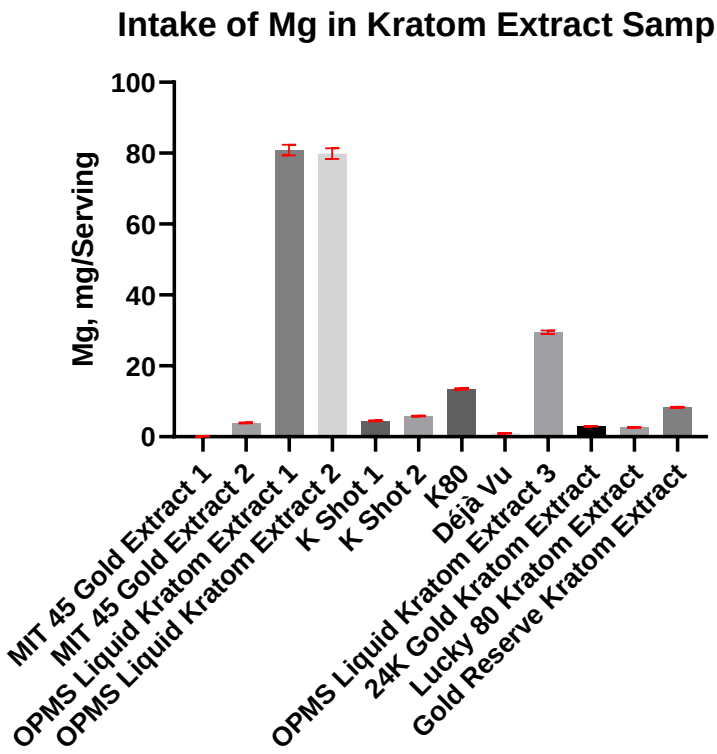

Figure 17: Intake of Mg in Kratom Extract Samples Based on Suggested Serving

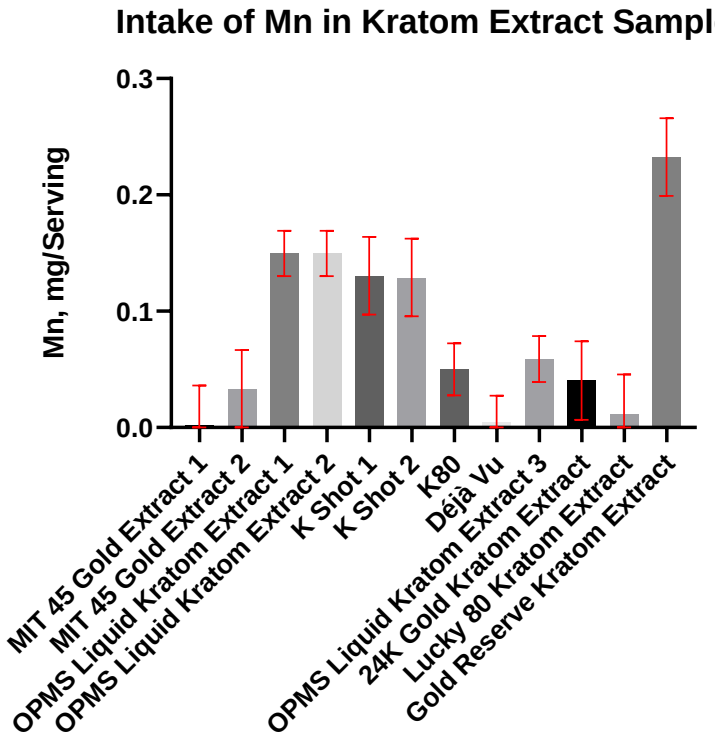

Figure 18: Intake of Mn in Kratom Extract Samples Based on Suggested Serving

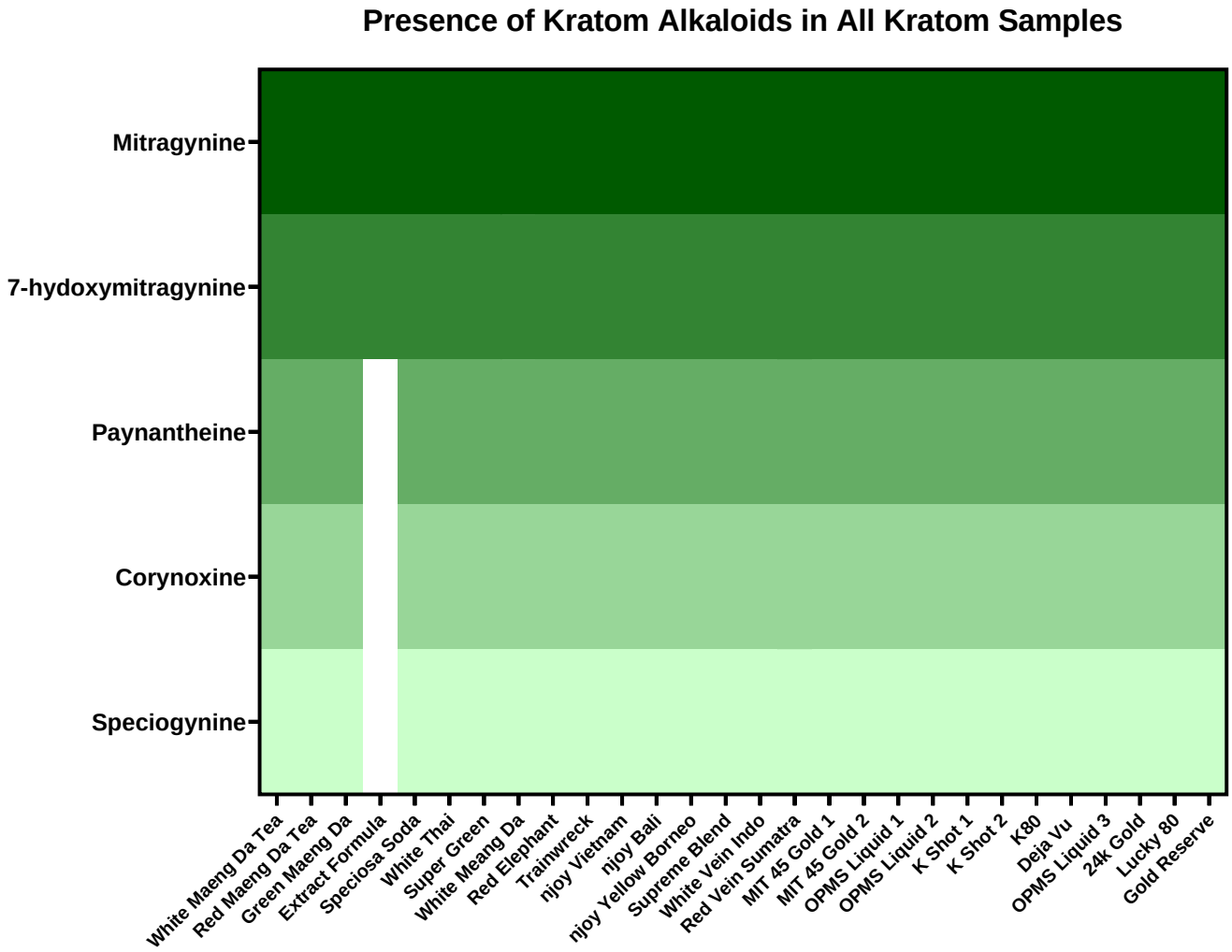

Figure 19: Heat Map of Kratom Alkaloids in Kratom Samples

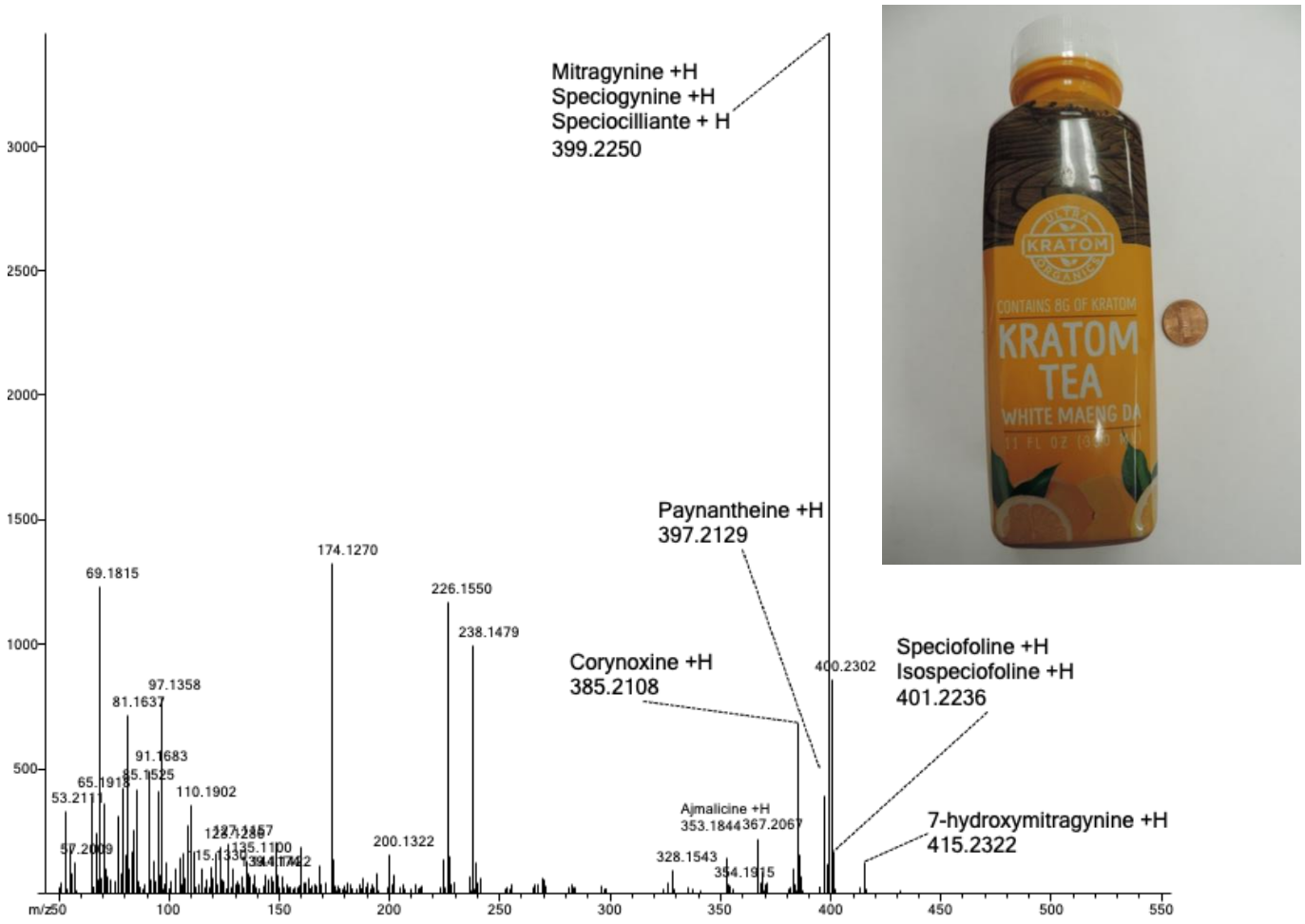

Figure 20: White Maeng Da Tea DART Mass Spectrum

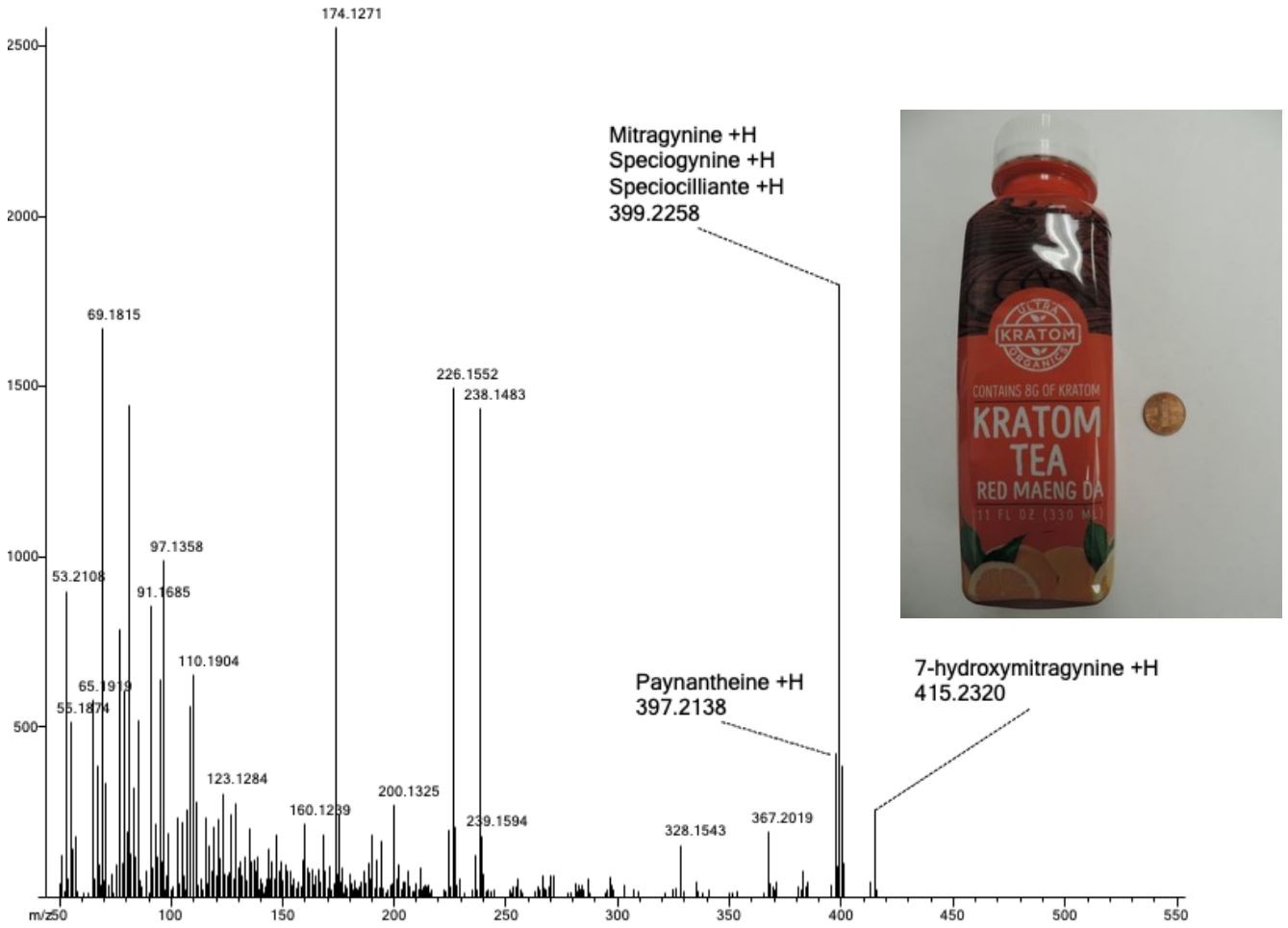

Figure 21: Red Maeng Da Kratom Tea DART Mass Spectrum

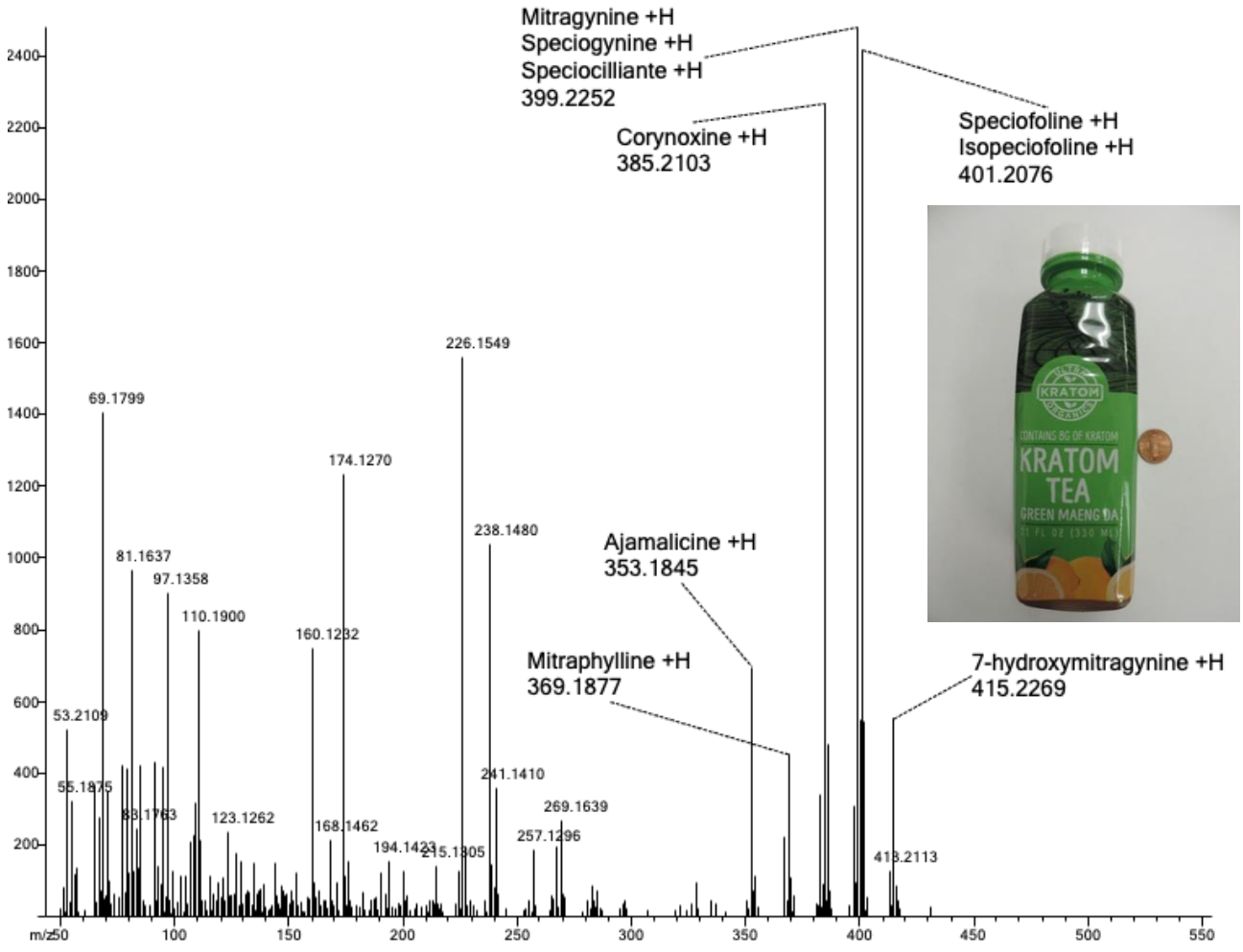

Figure 22: Green Kratom Tea DART Mass Spectrum

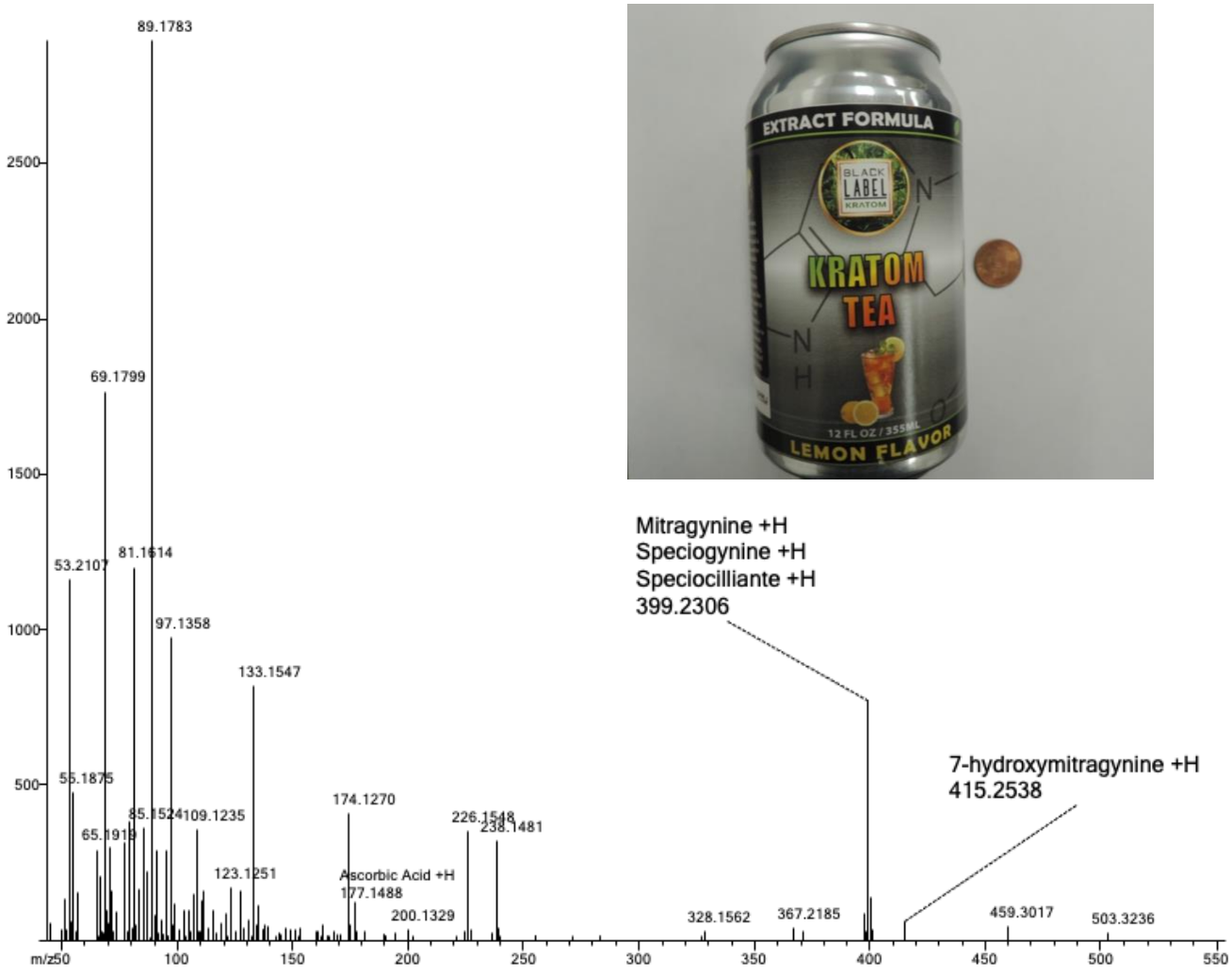

Figure 23: Kratom Tea Lemon Flavor DART Mass Spectrum

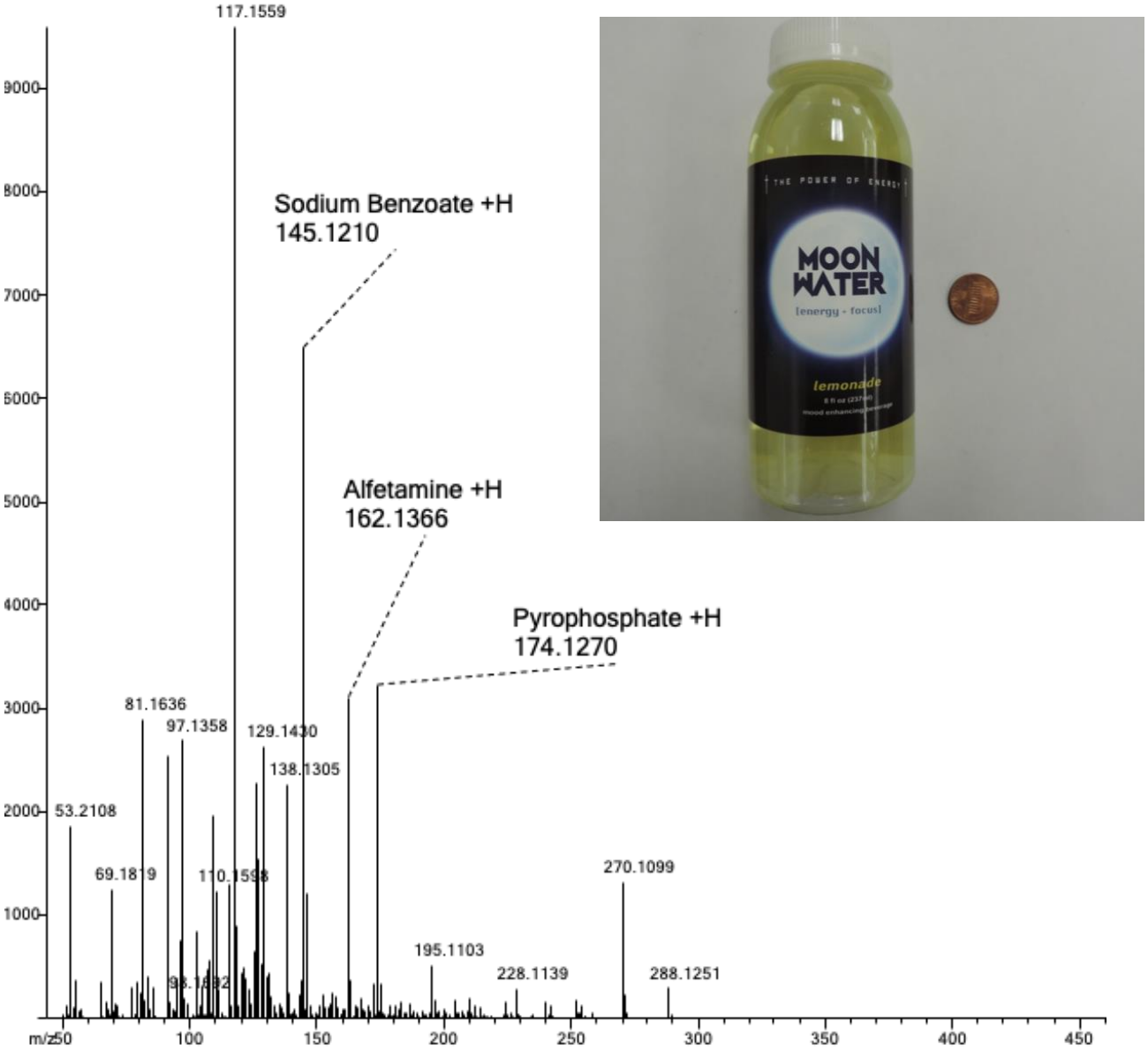

Figure 24: Moon Water DART Mass Spectrum

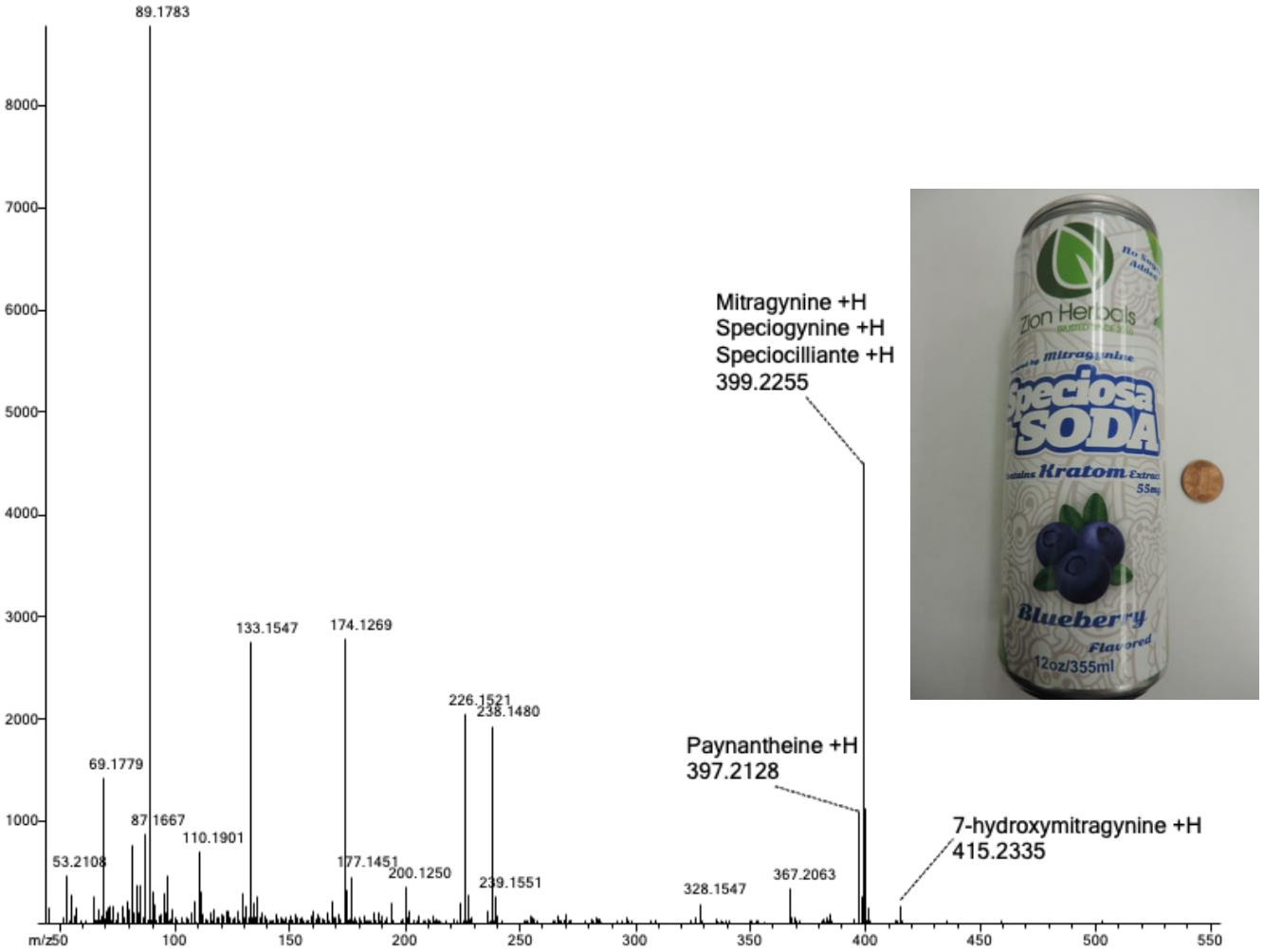

Figure 25: Speciosa Soda DART Mass Spectrum

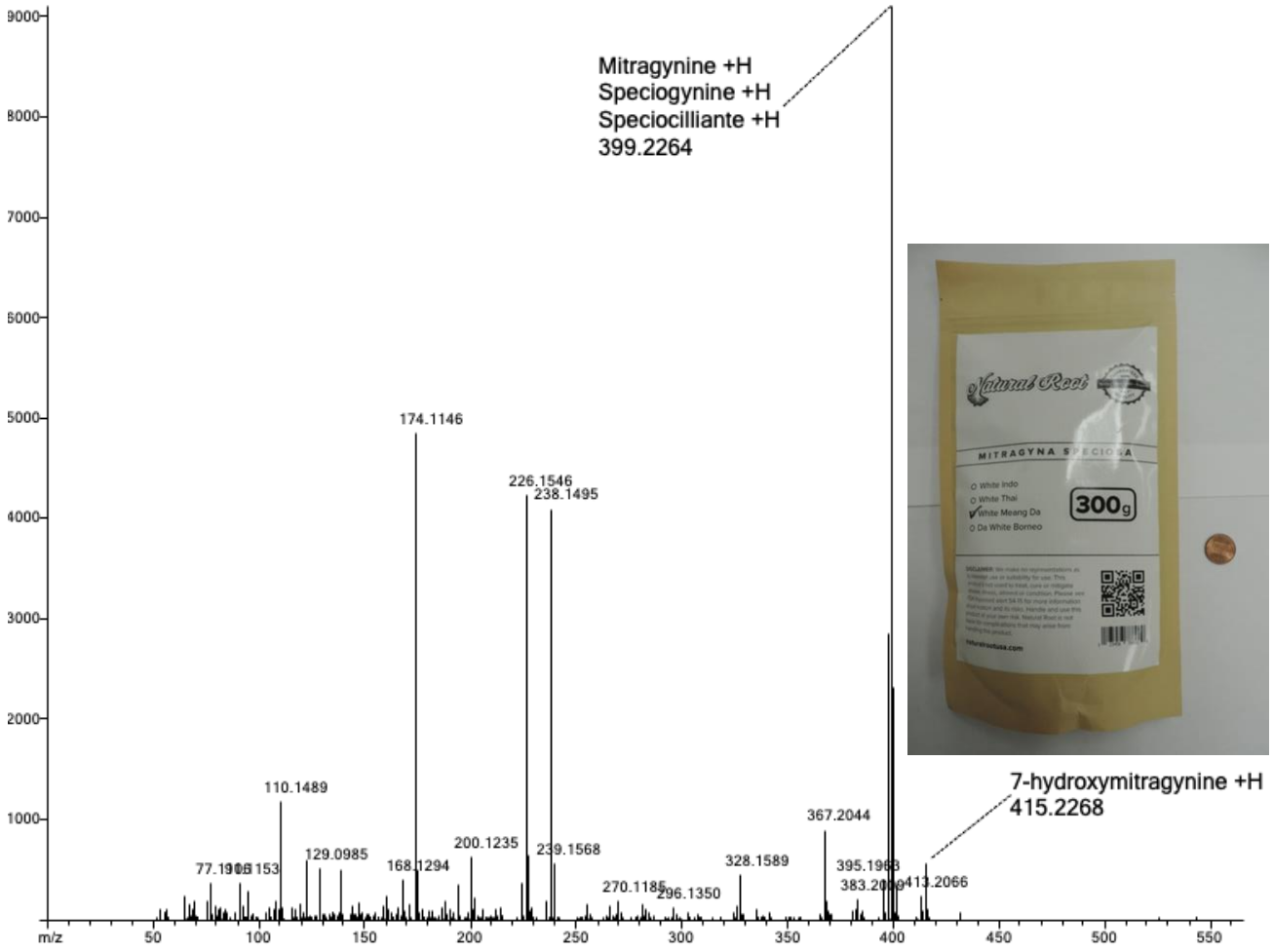

Figure 26: White Maeng Da Powder DART Mass Spectrum

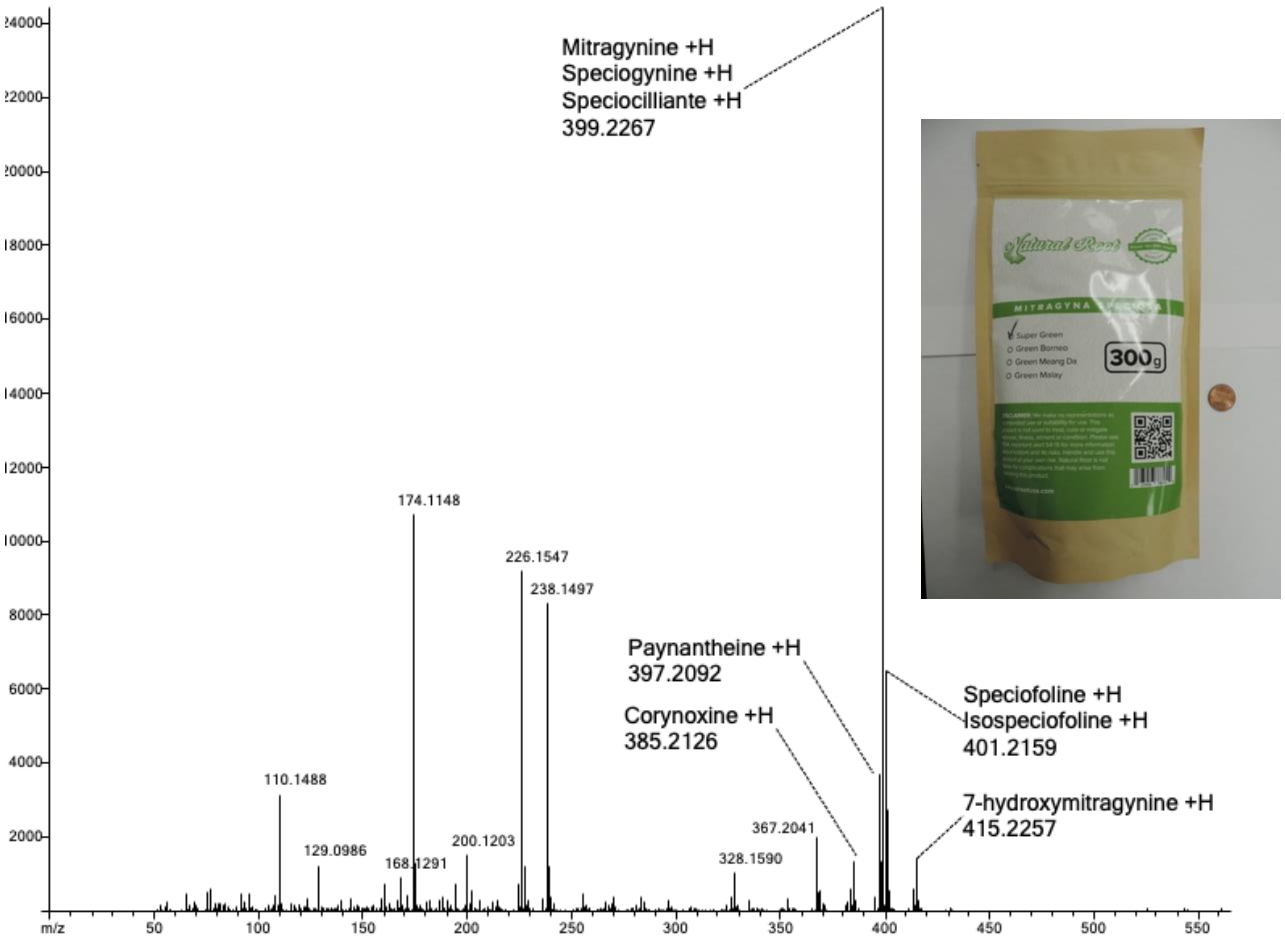

Figure 27: Kratom Super Green Powder DART Mass Spectrum

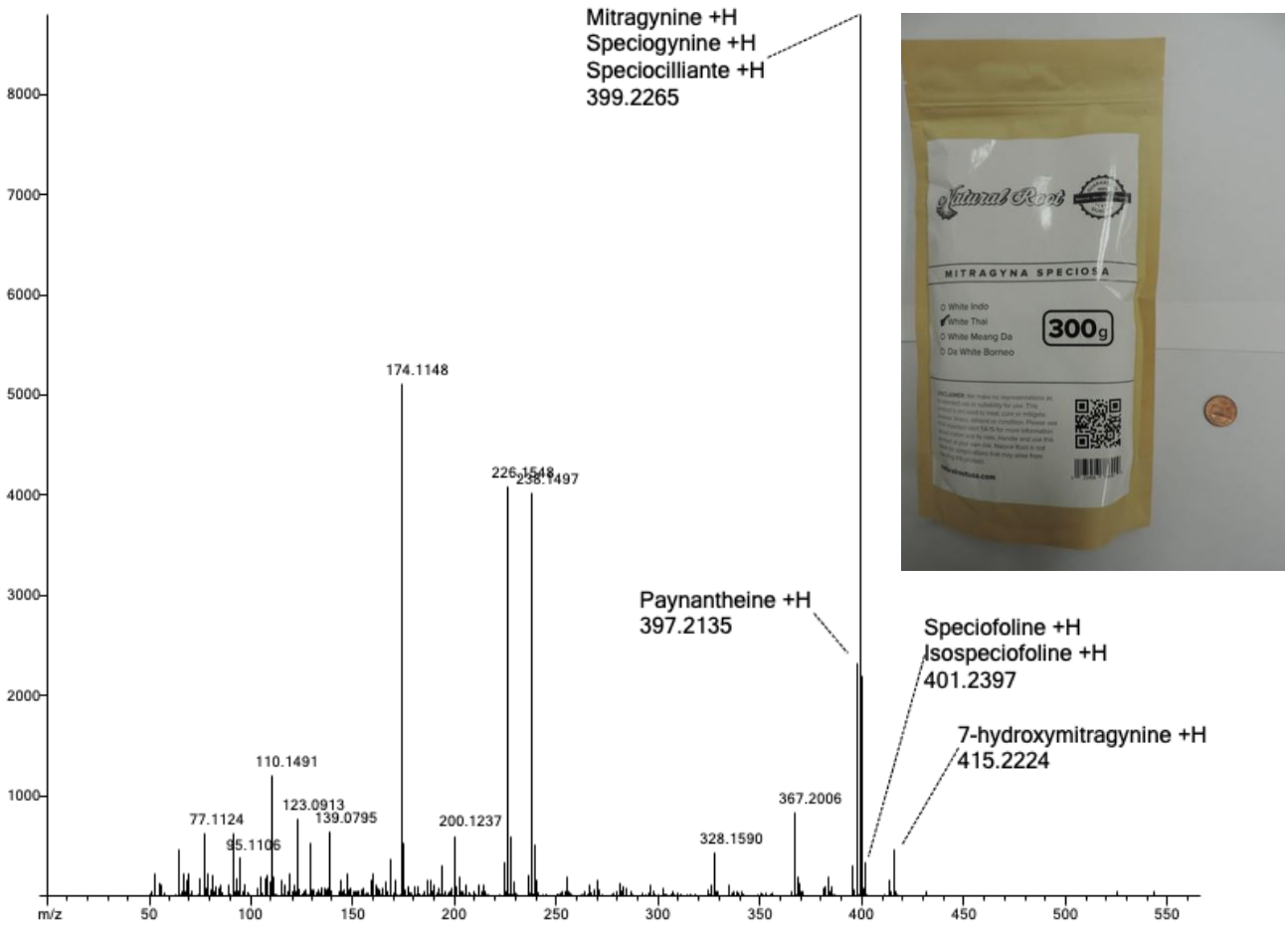

Figure 28: White Thai Kratom Powder DART Mass Spectrum

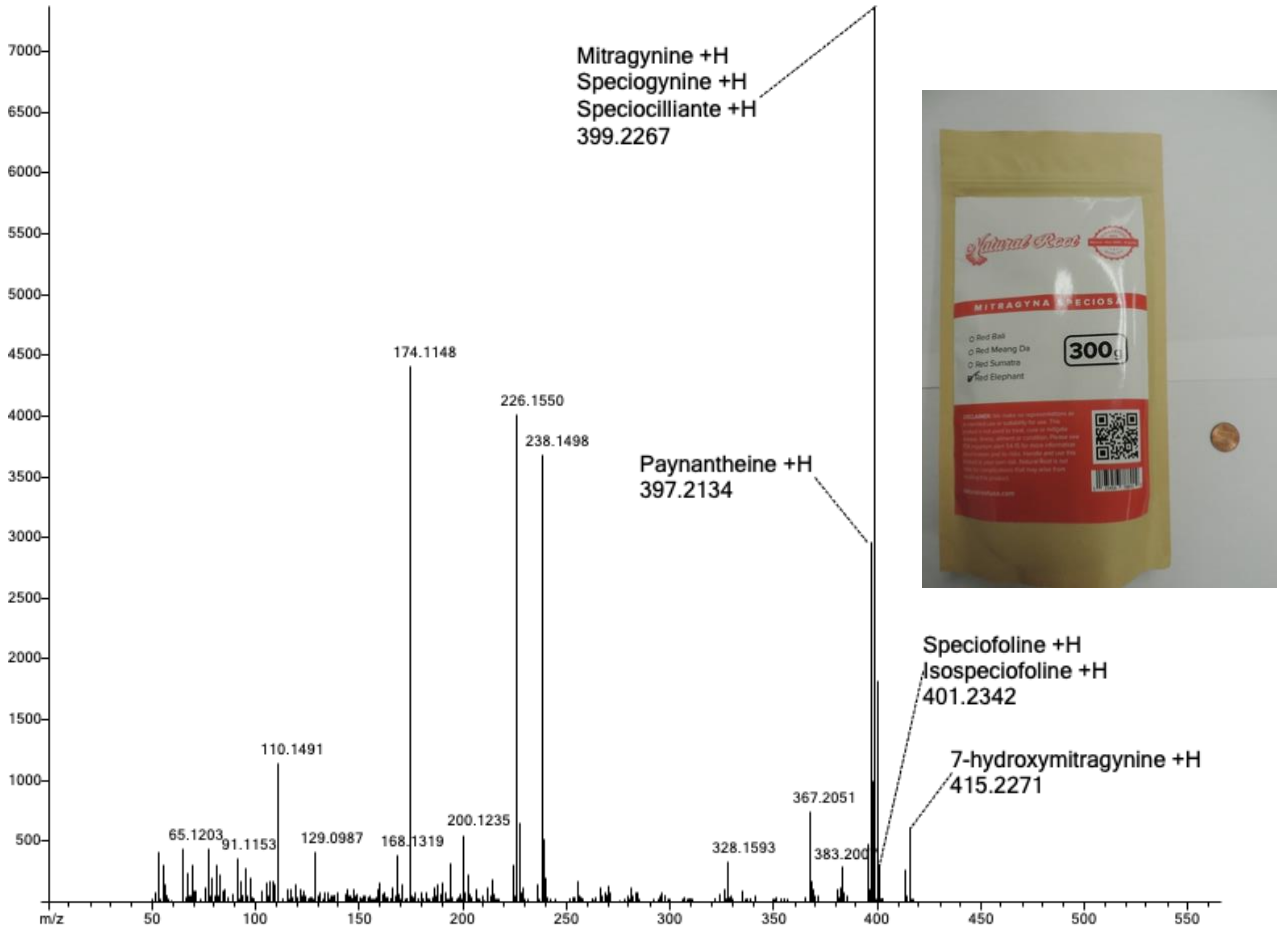

Figure 29: Red Elephant Kratom Powder DART Mass Spectrum

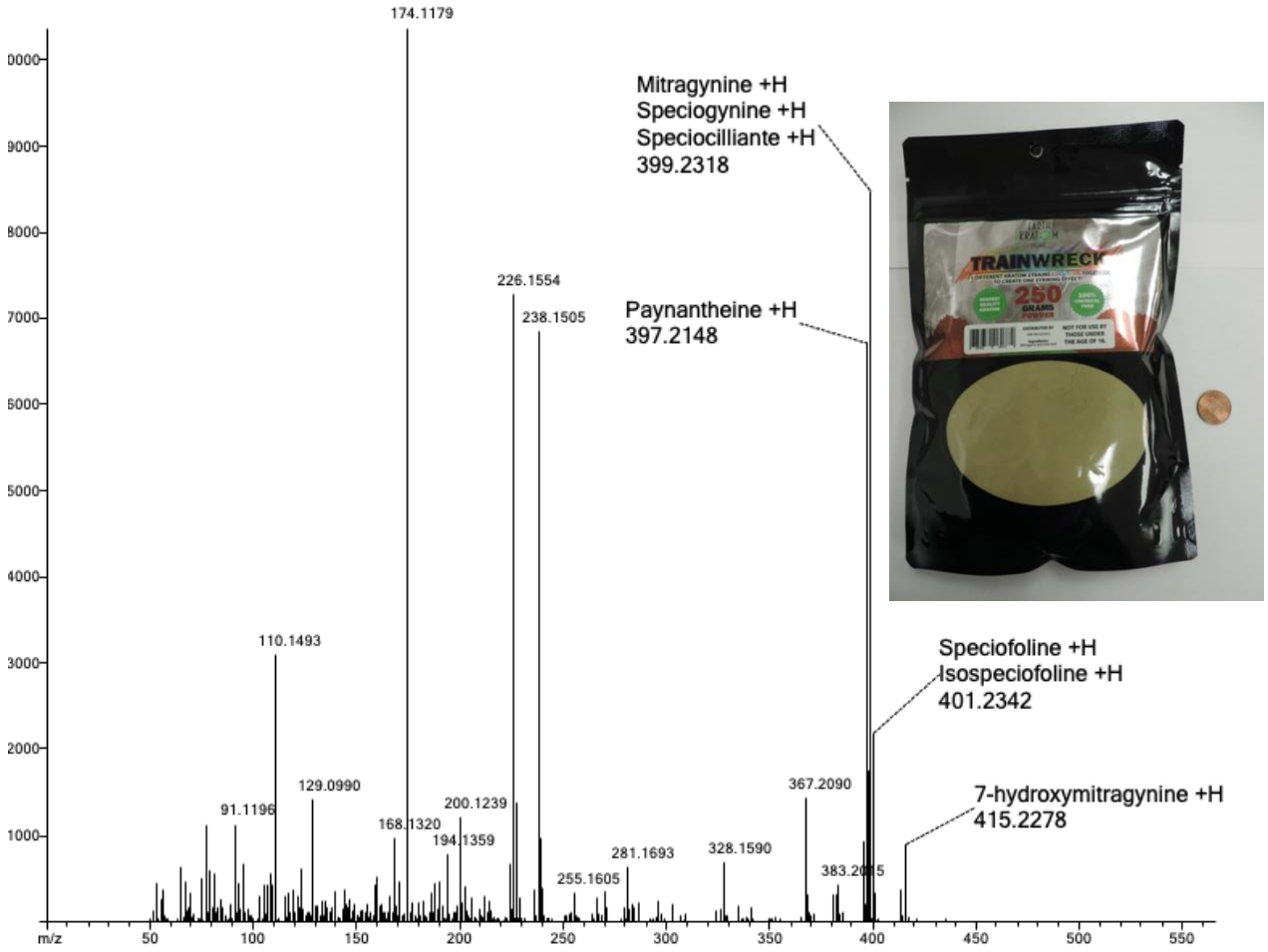

Figure 30: Trainwreck Kratom Powder DART Mass Spectrum

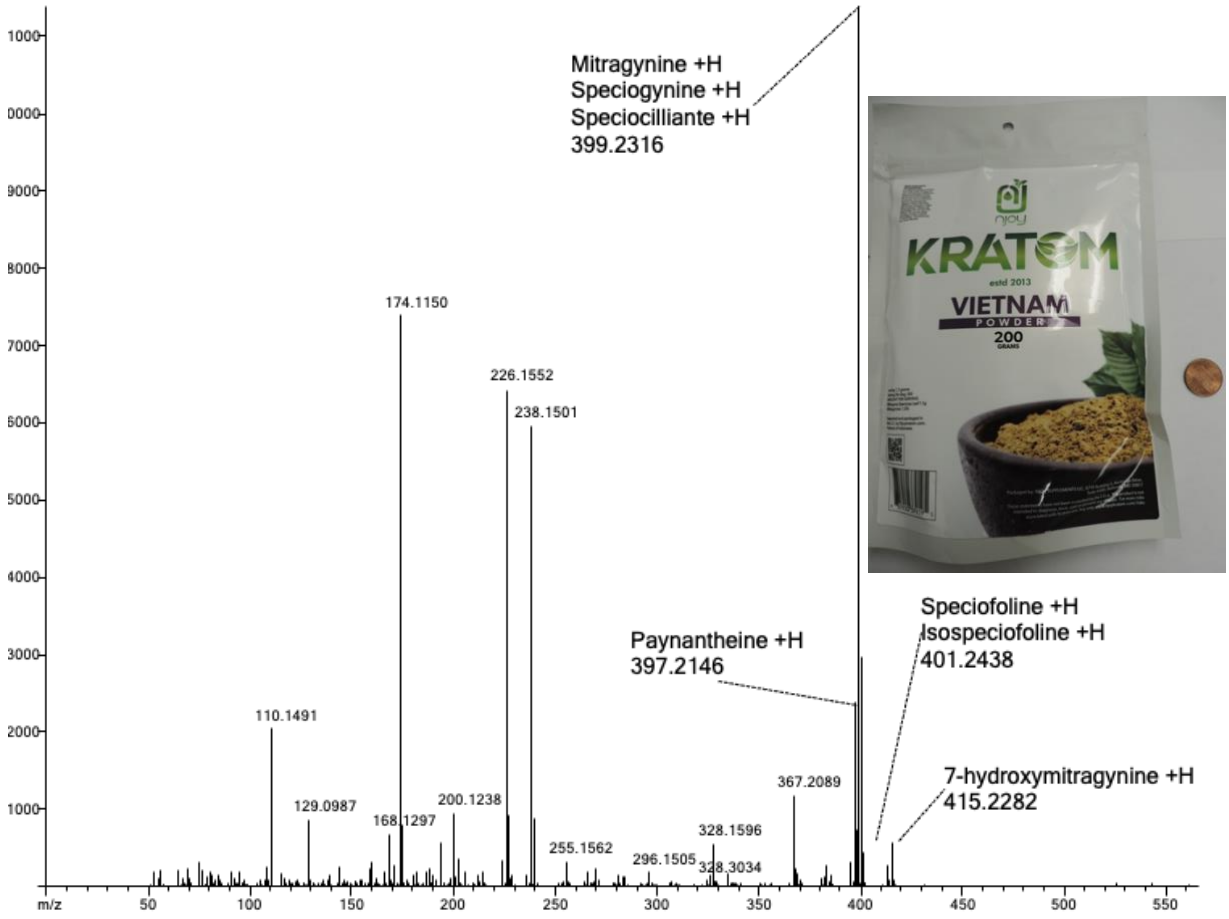

Figure 31: Vietnam Kratom Powder DART Mass Spectrum

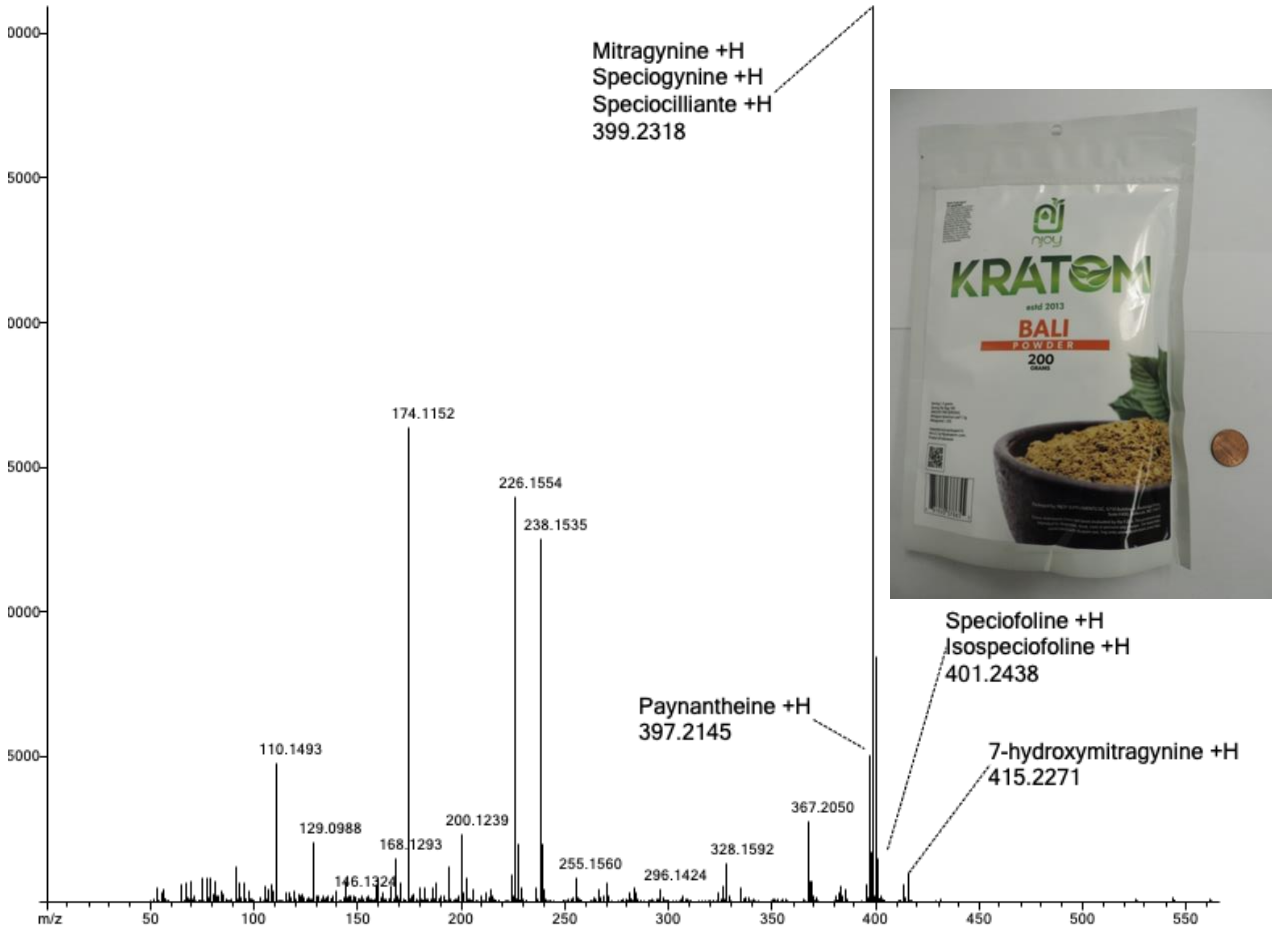

Figure 32: Kratom Bali Powder DART Mass Spectrum

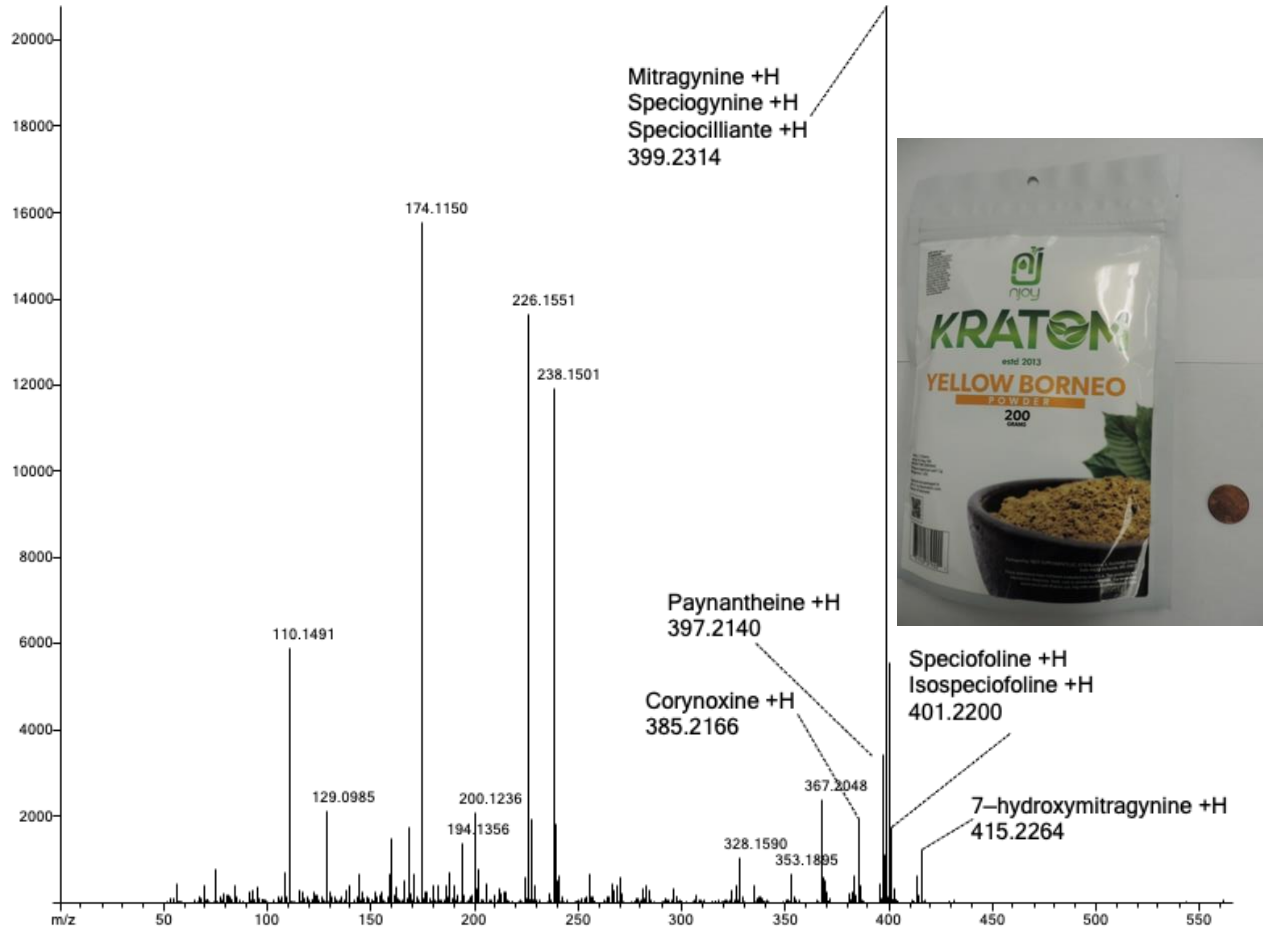

Figure 33: Yellow Borneo Kratom Powder DART Mass Spectrum

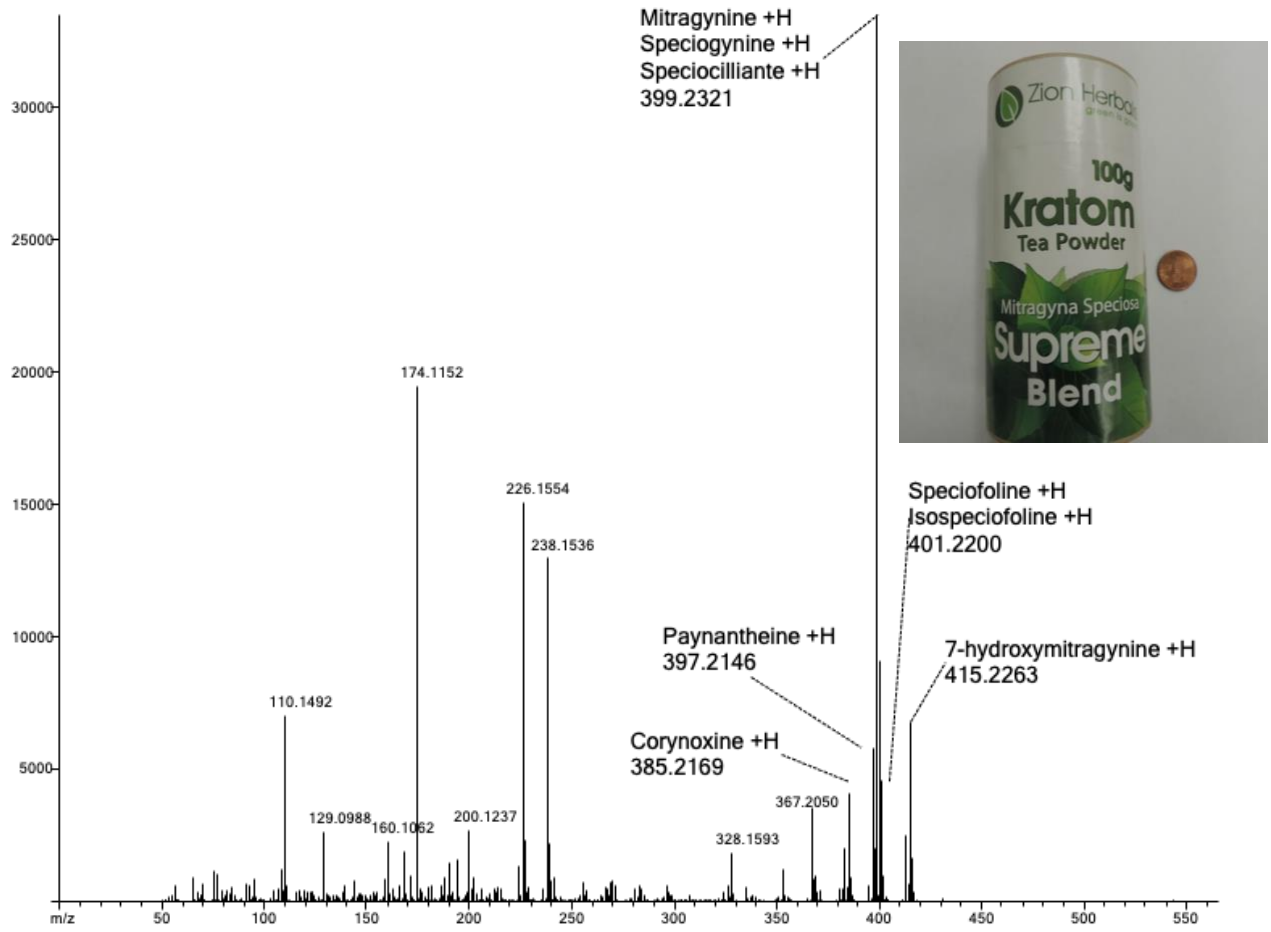

Figure 34: Kratom Supreme Blend Powder DART Mass Spectrum

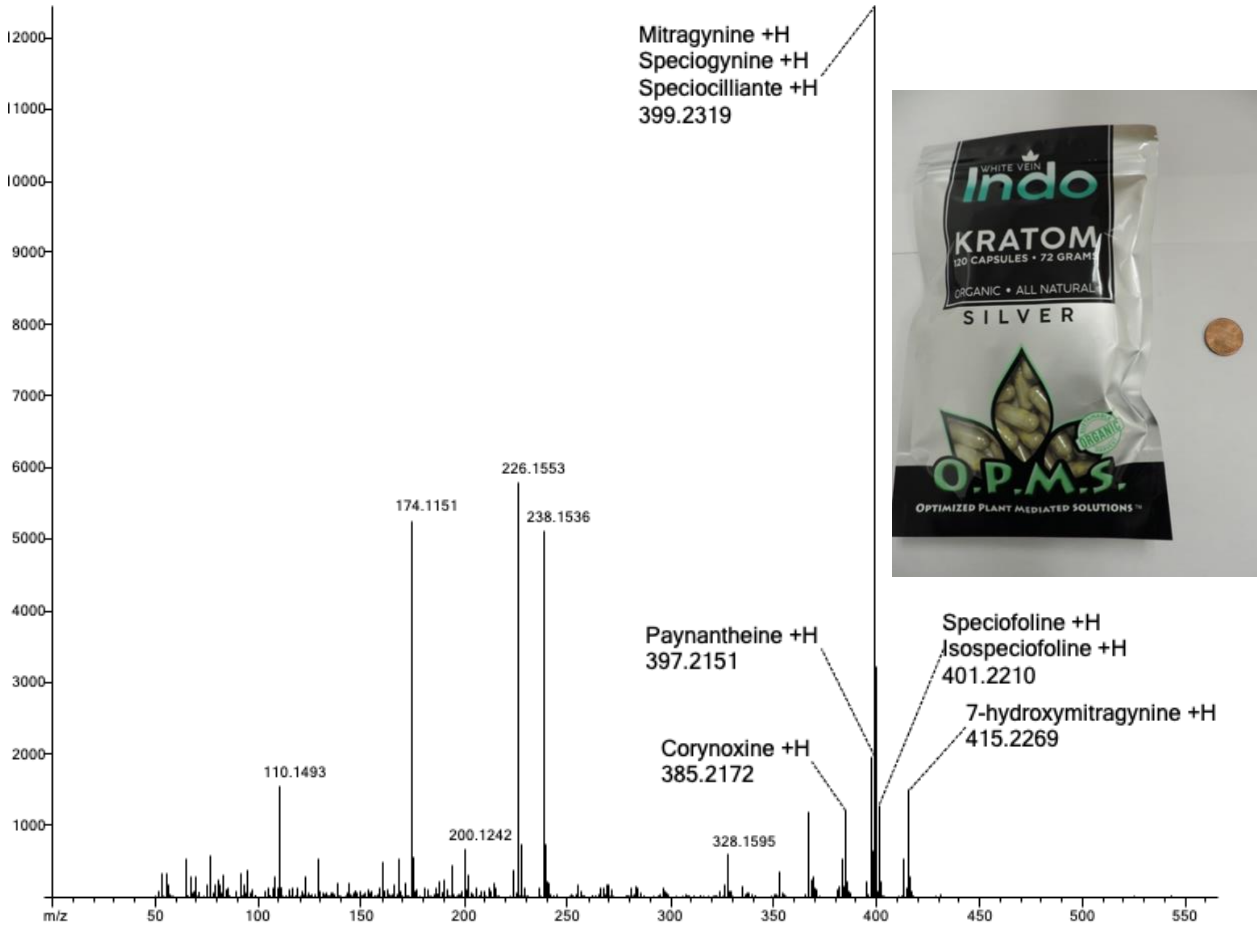

Figure 35: White Vein Indo Kratom Capsules DART Mass Spectrum

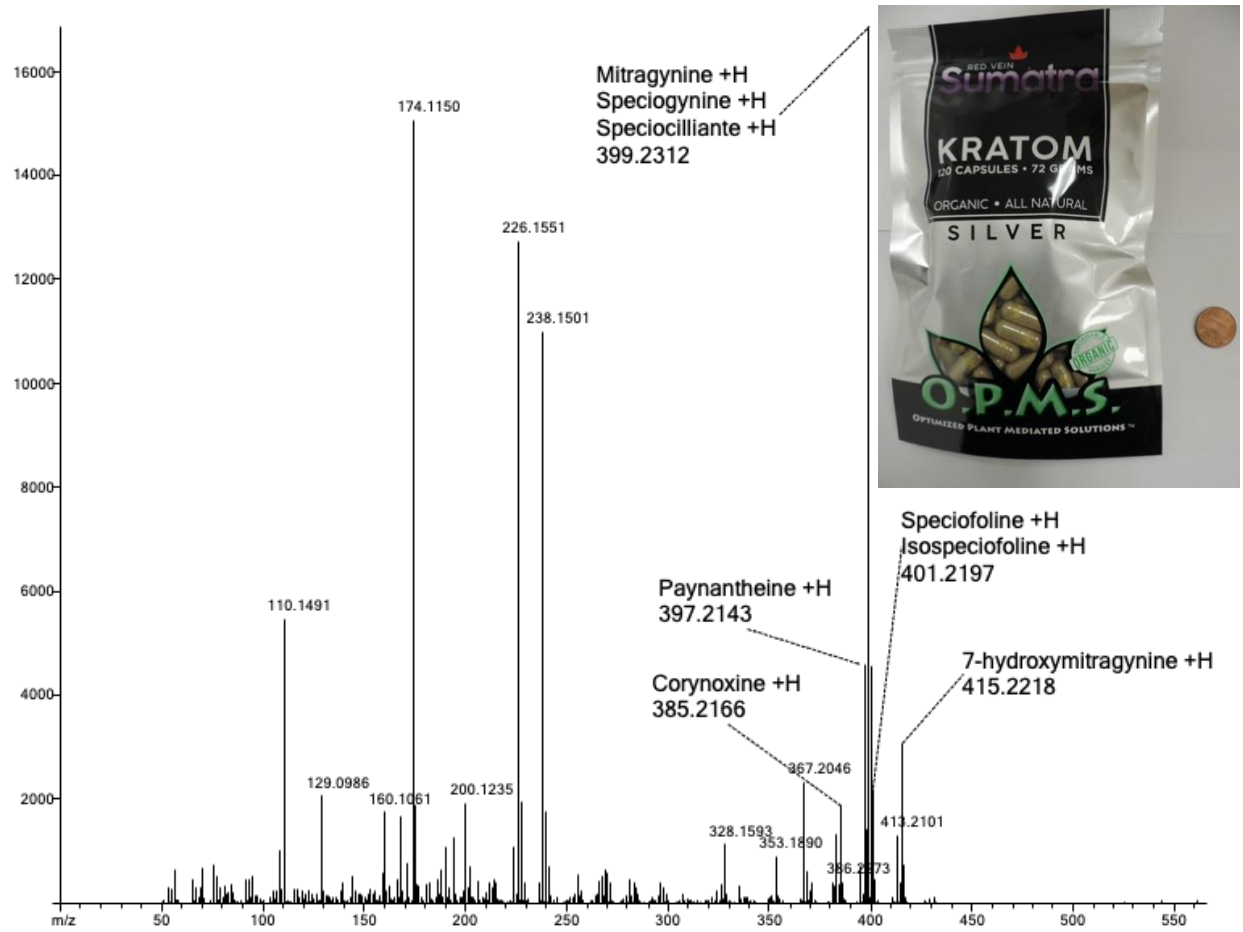

Figure 36: Red Vein Sumatra Kratom Capsules DART Mass Spectrum

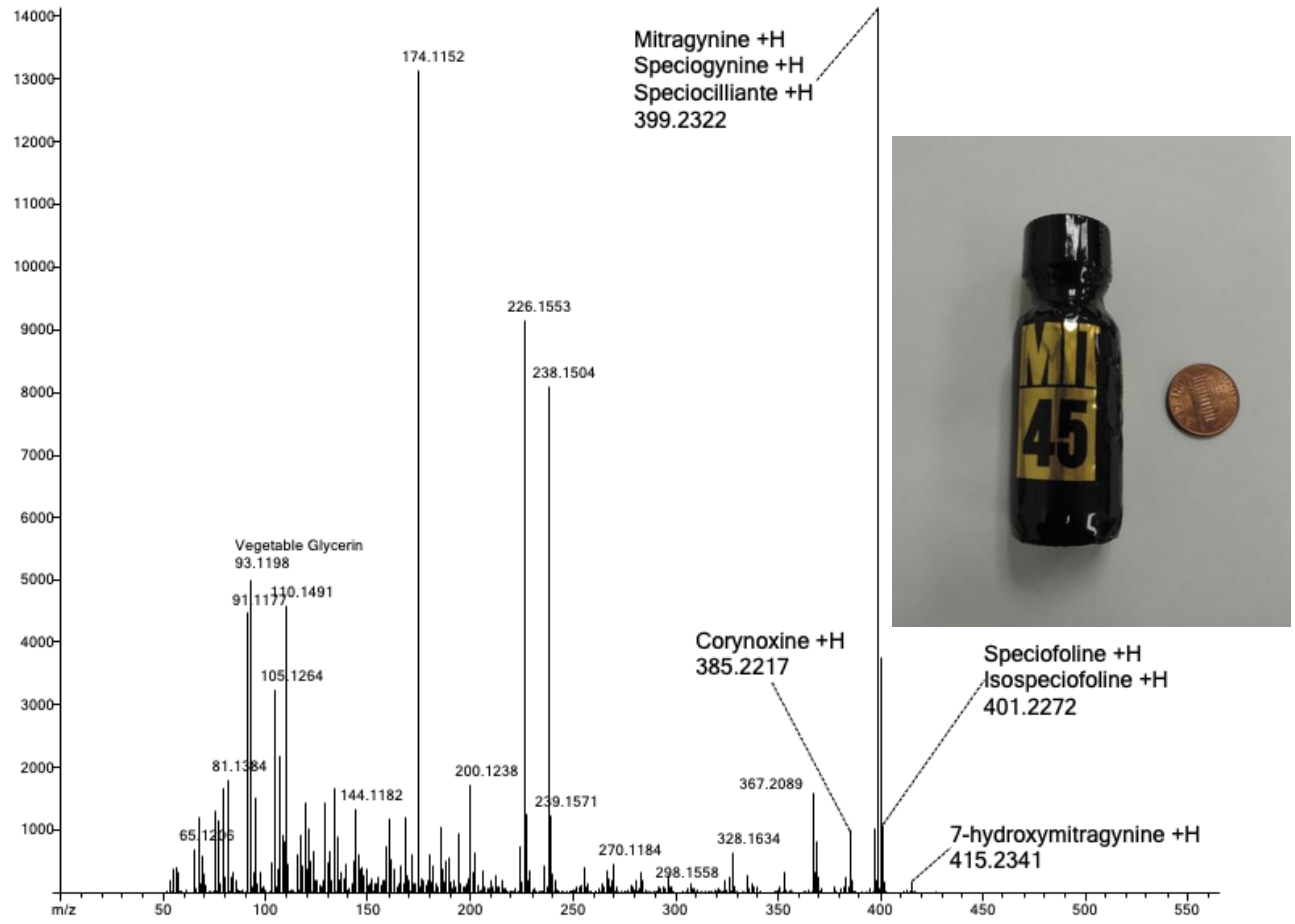

Figure 37: MIT 45 Kratom Extract DART Mass Spectrum

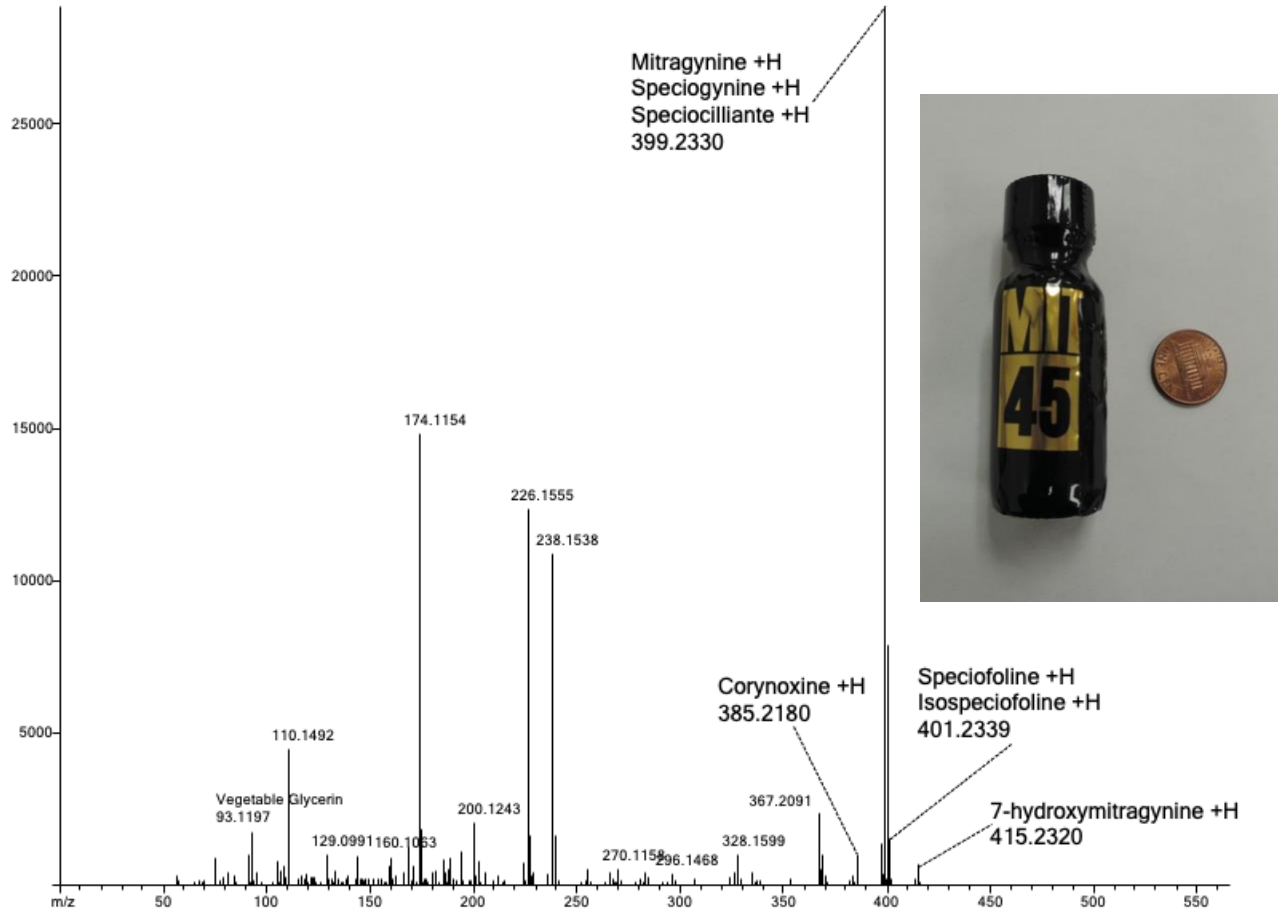

Figure 38: MIT 45 Kratom Extract DART Mass Spectrum

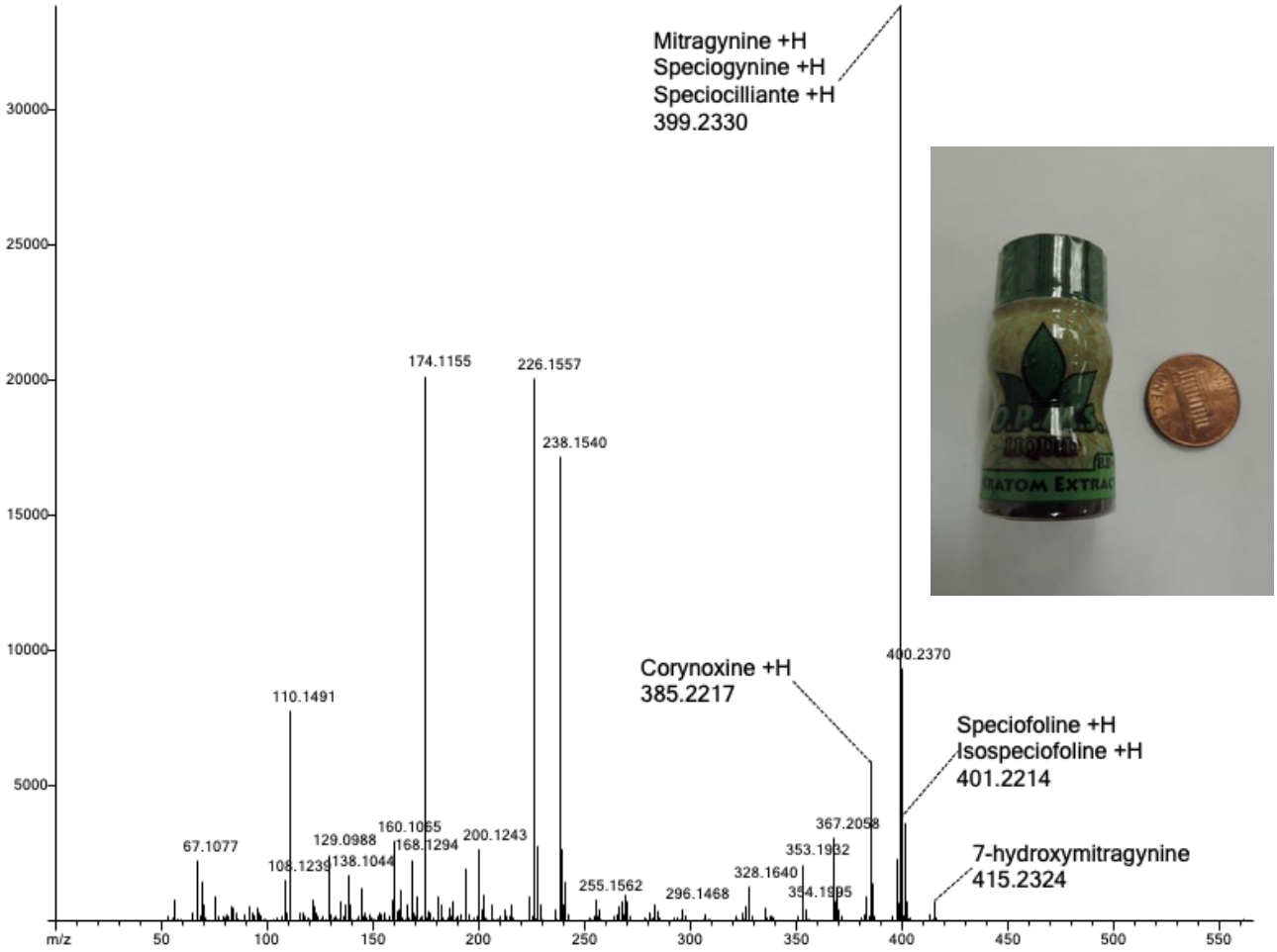

Figure 39: OPMS Kratom Extract DART Mass Spectrum

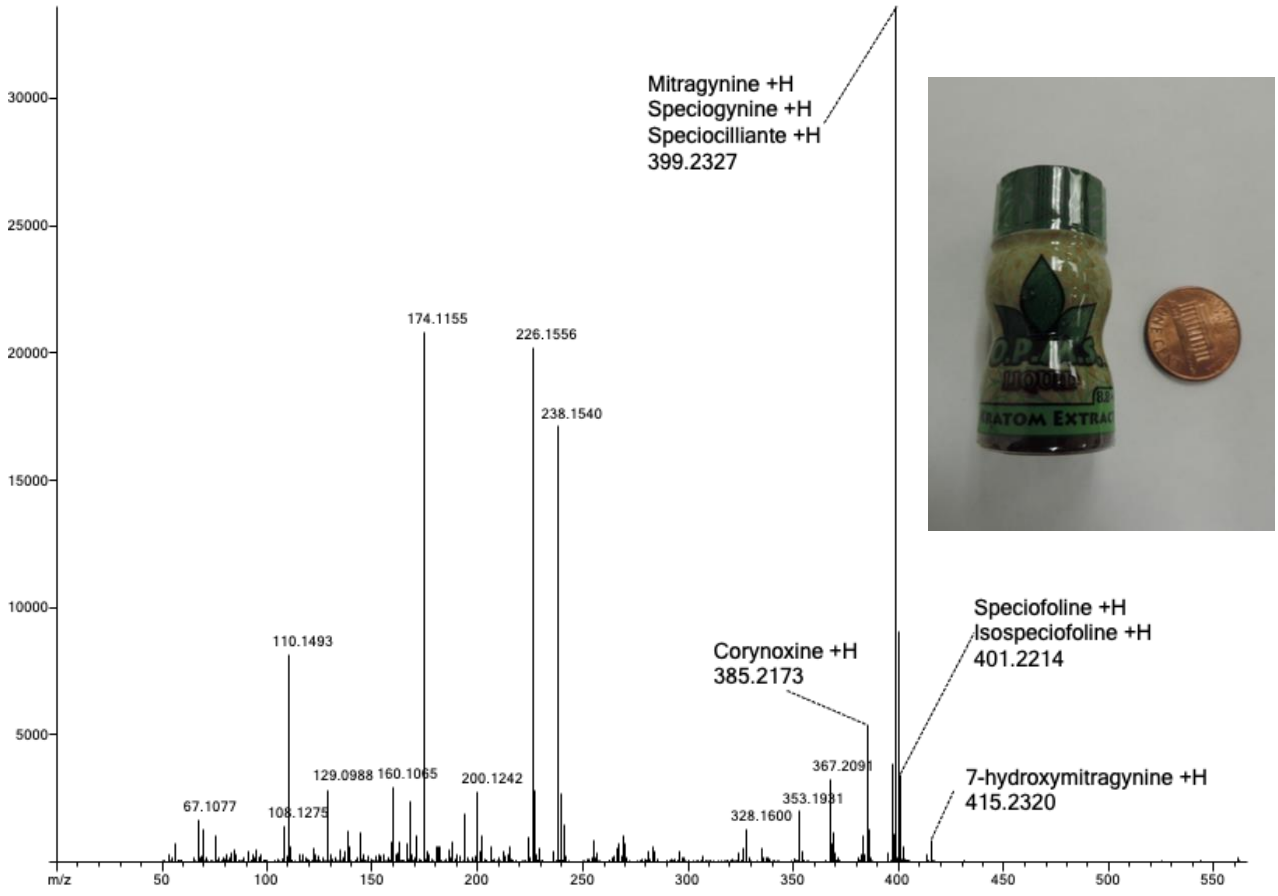

Figure 40: OPMS Kratom Extract DART Mass Spectrum

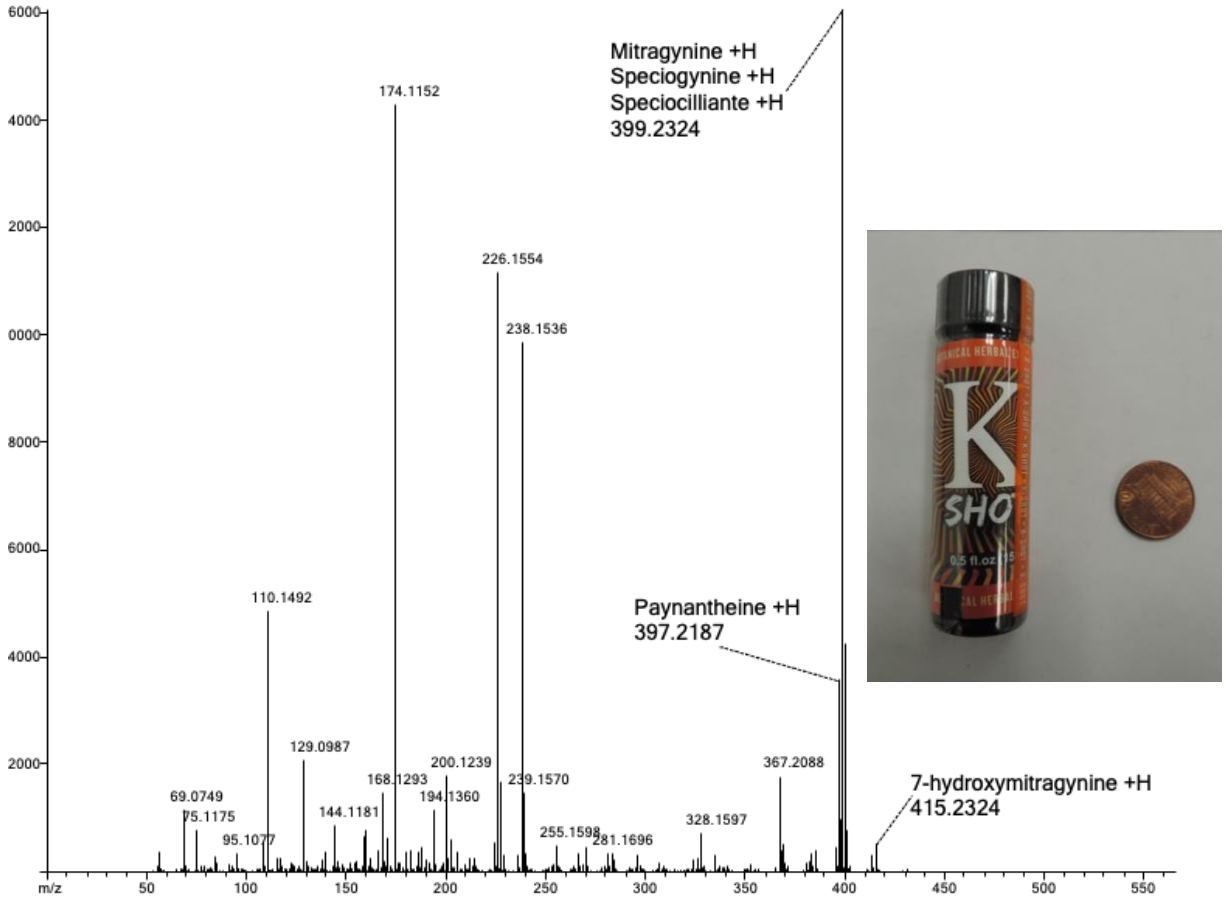

Figure 41: K Shot Kratom Extract DART Mass Spectrum

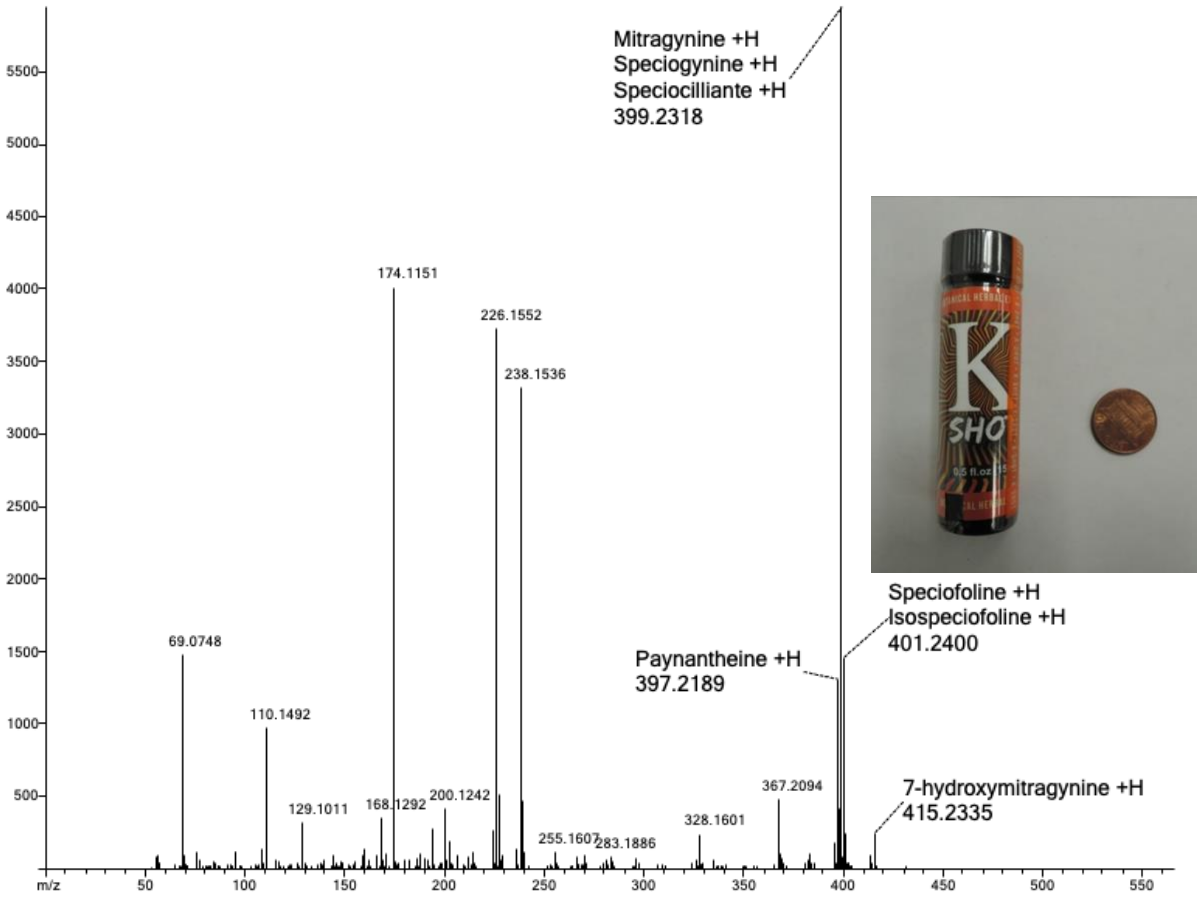

Figure 42: K Shot Kratom Extract DART Mass Spectrum

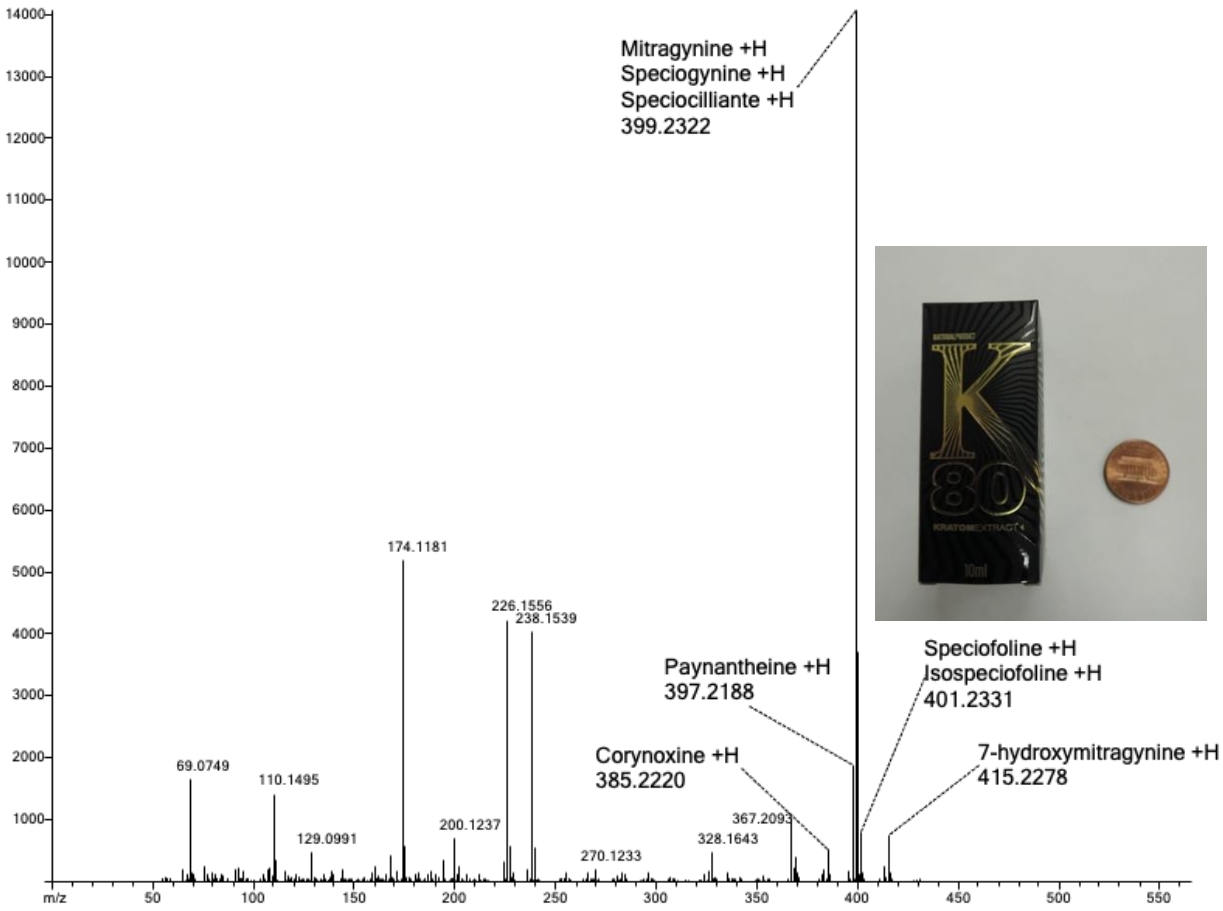

Figure 43: K Shot Kratom Extract DART Mass Spectrum

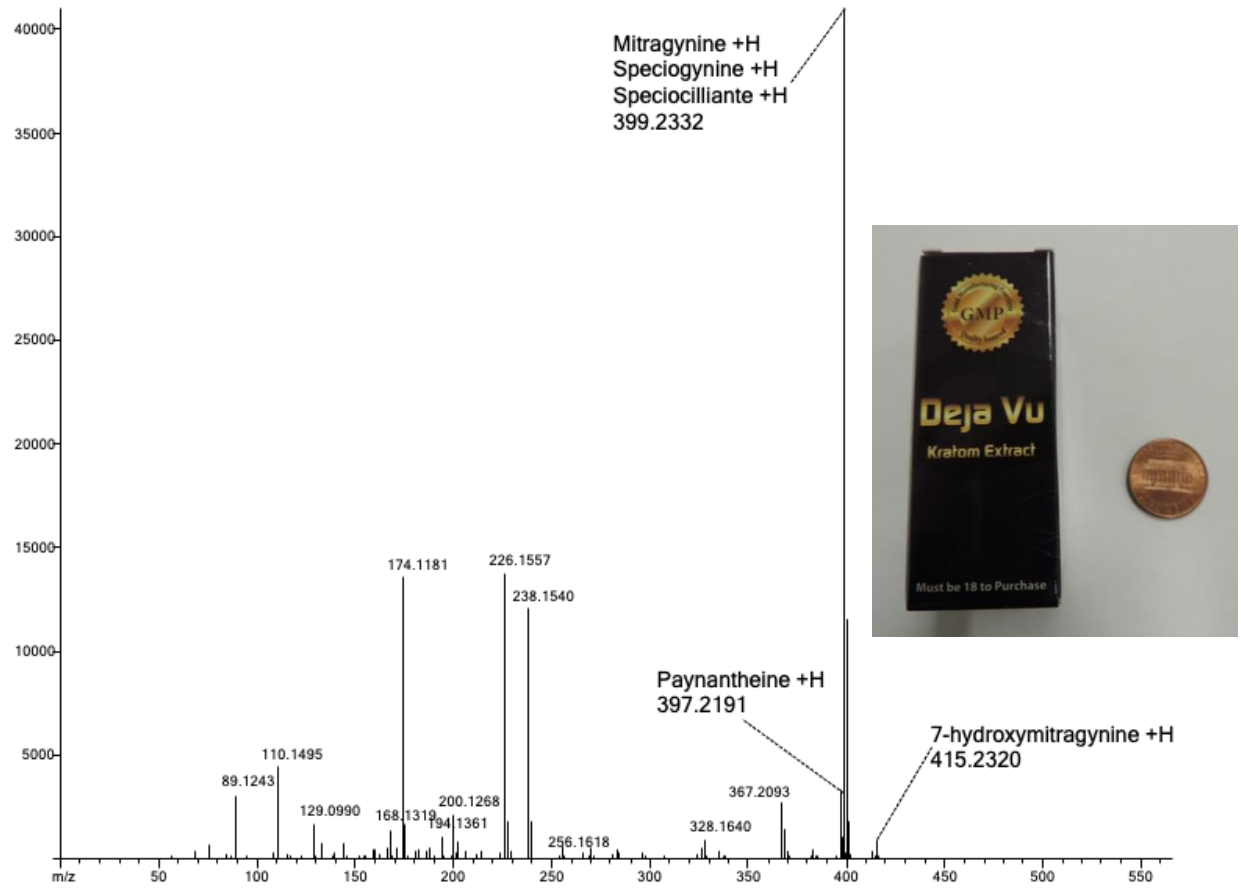

Figure 44: Déjà vu Kratom Extract DART Mass Spectrum

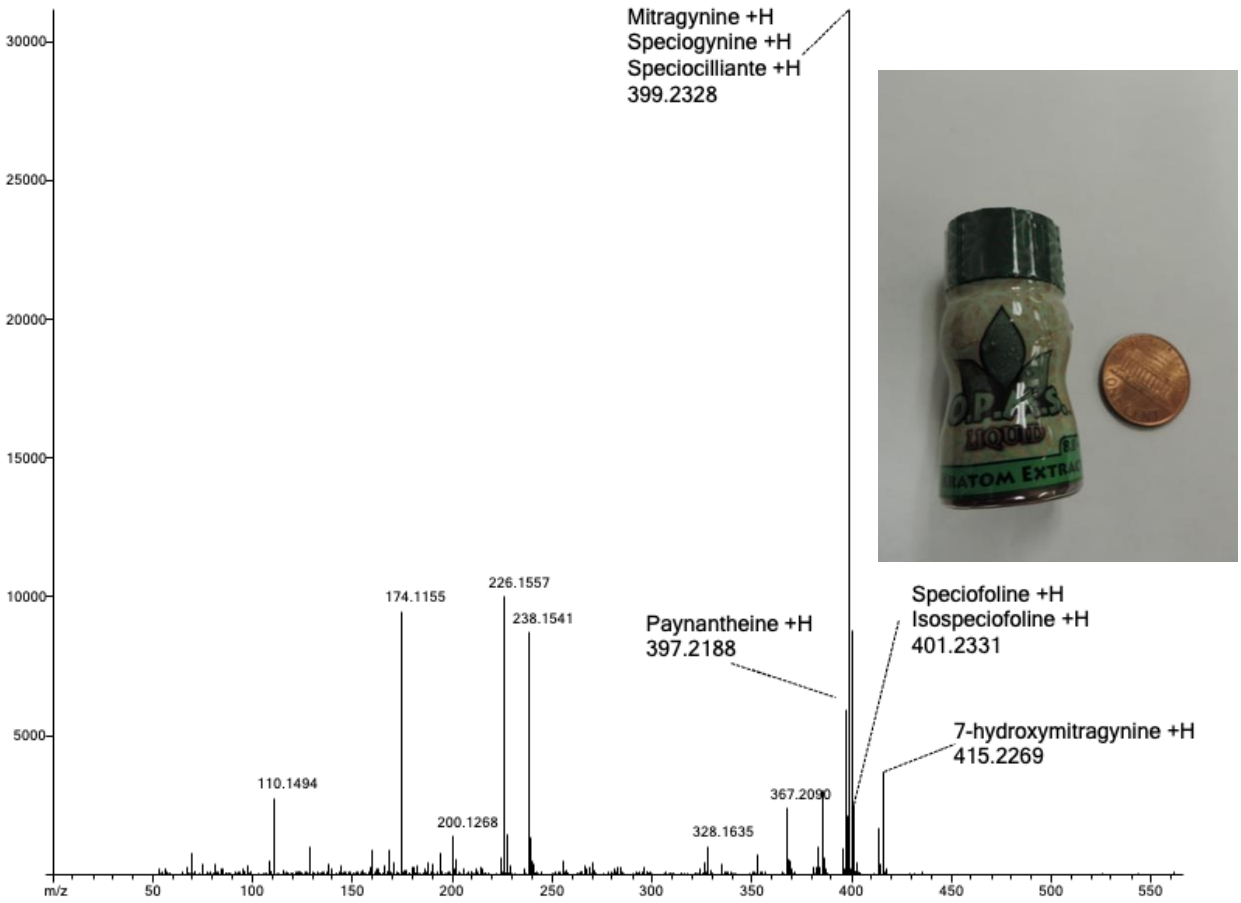

Figure 45: OPMS Kratom Extract DART Mass Spectrum

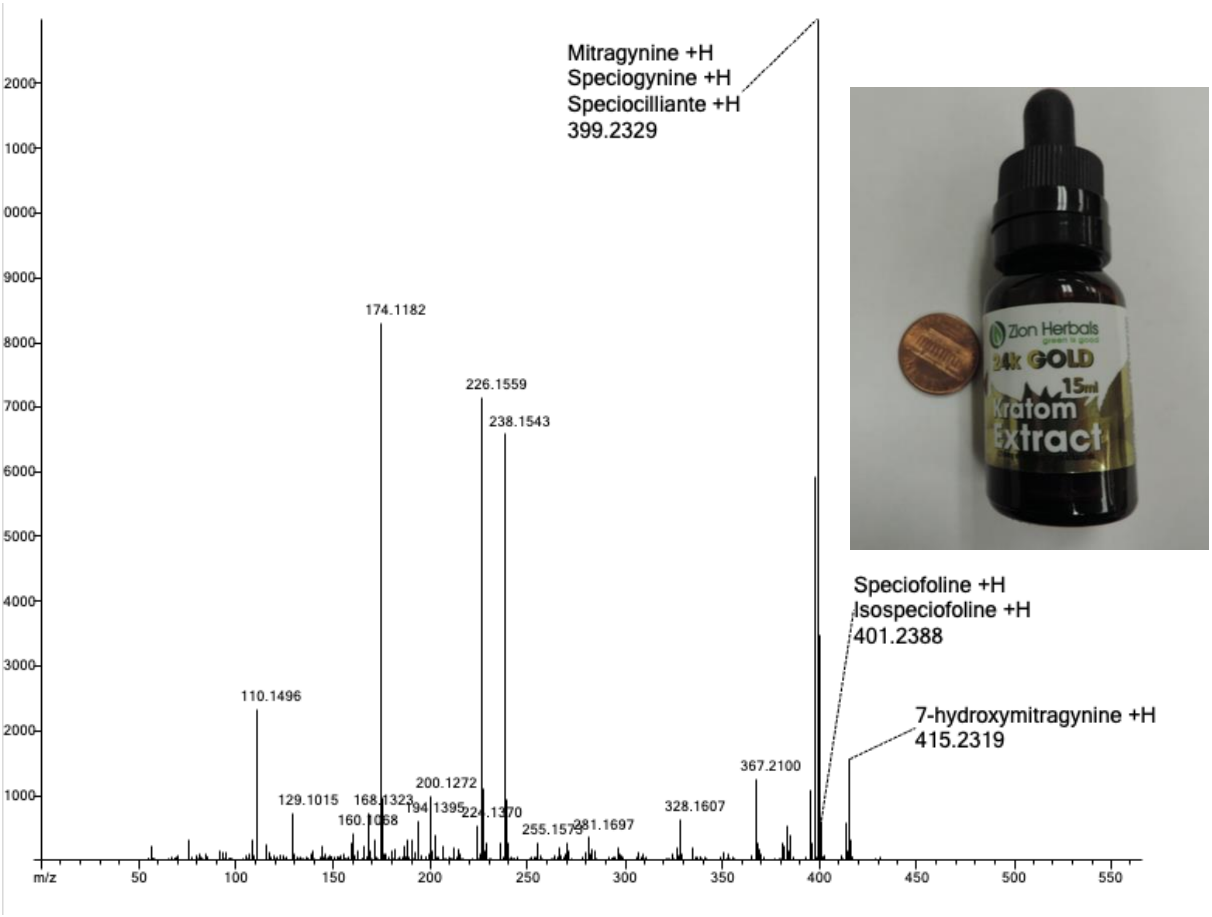

Figure 46: 24K Gold Kratom Extract DART Mass Spectrum

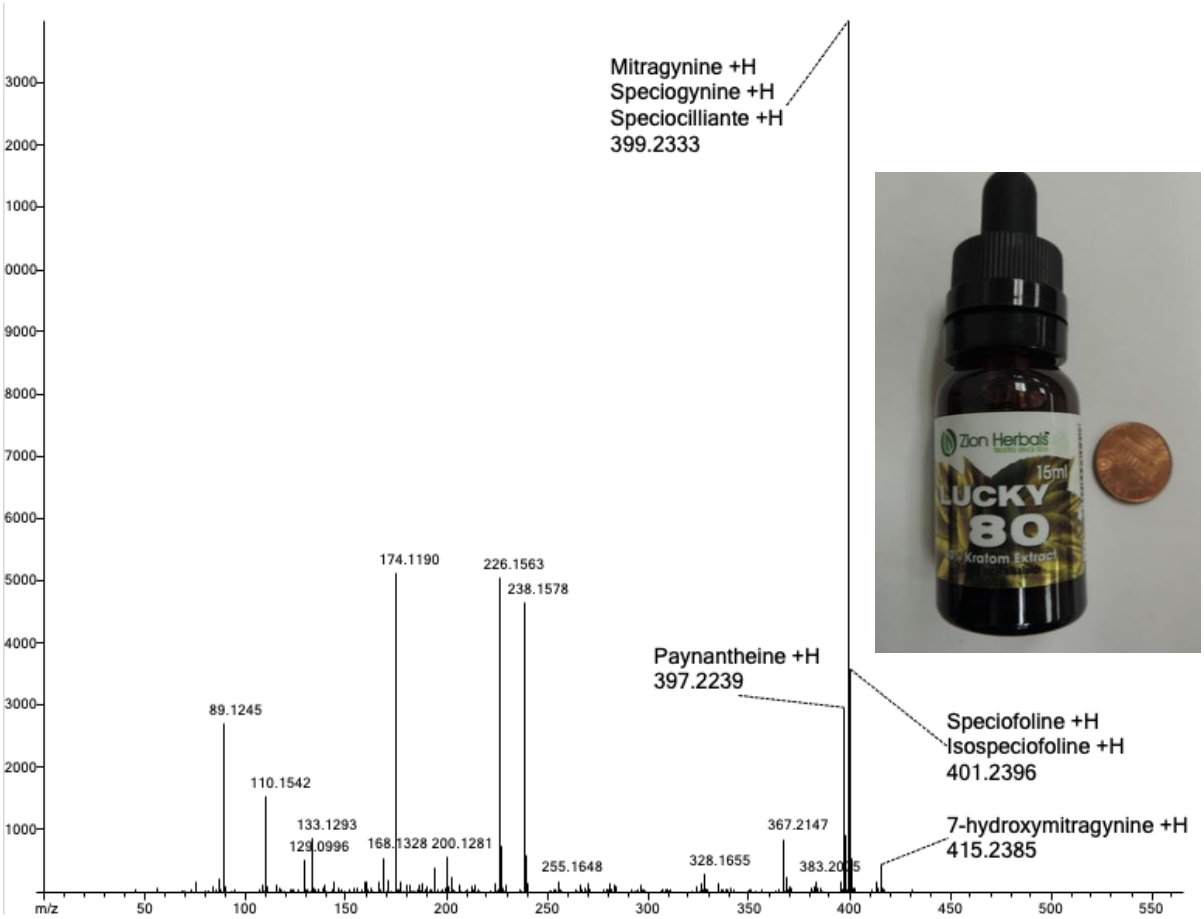

Figure 47: Lucky 80 Kratom Extract DART Mass Spectrum

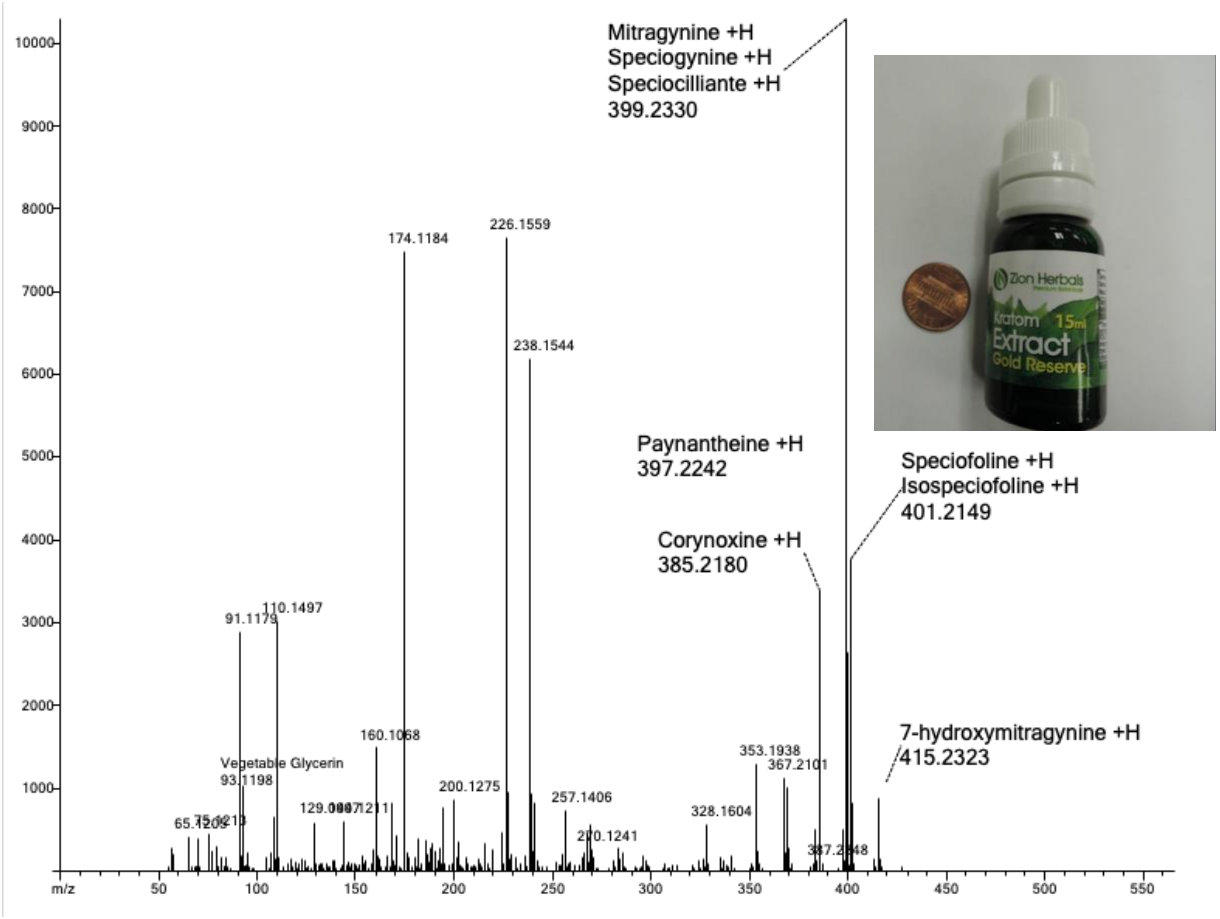

Figure 48: Gold Reserve Kratom Extract DART Mass Spectrum

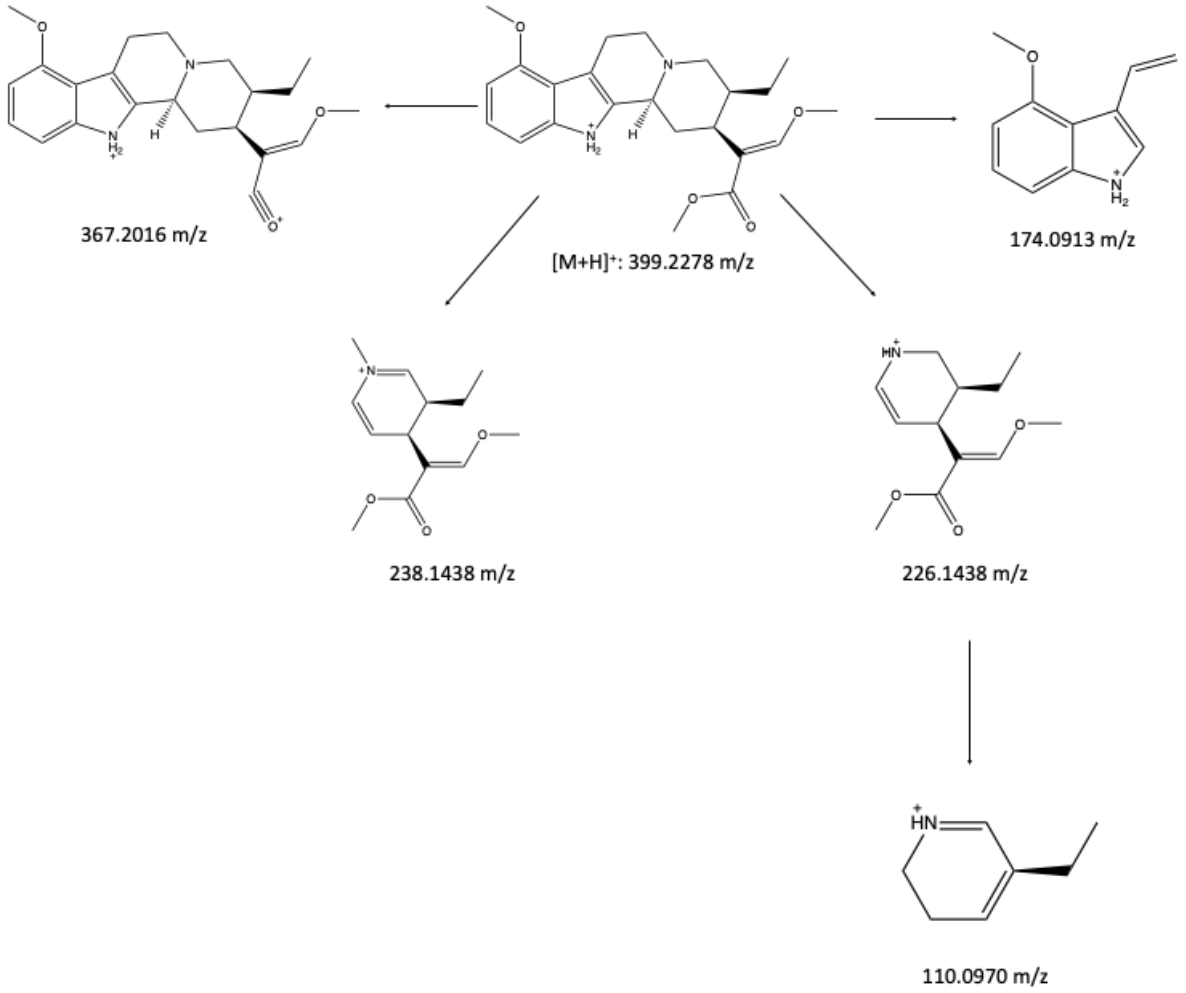

Figure 49: Fragmentation of Mitragynine

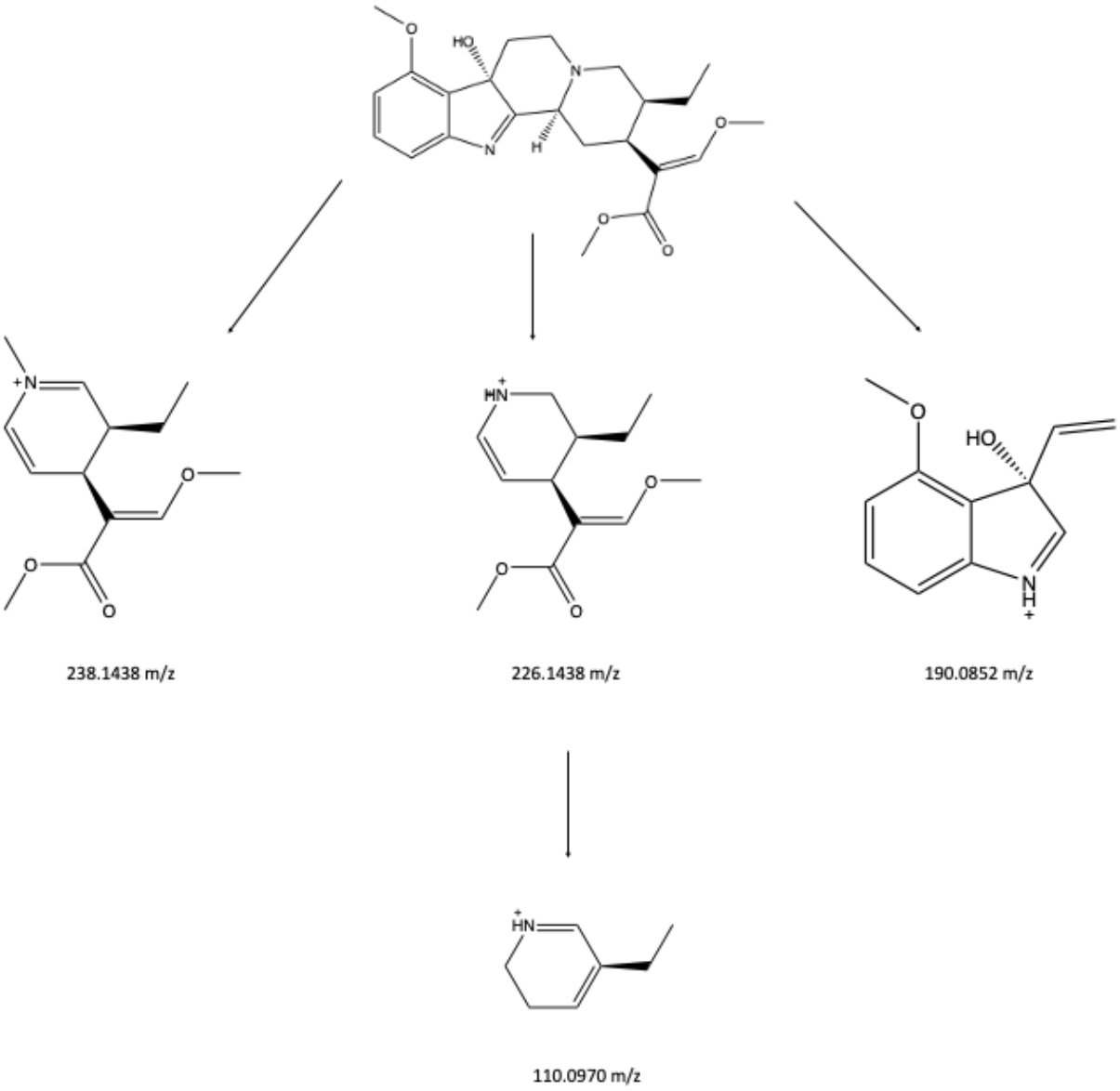

Figure 50: Fragmentation of 7-hydroxymtiragynine

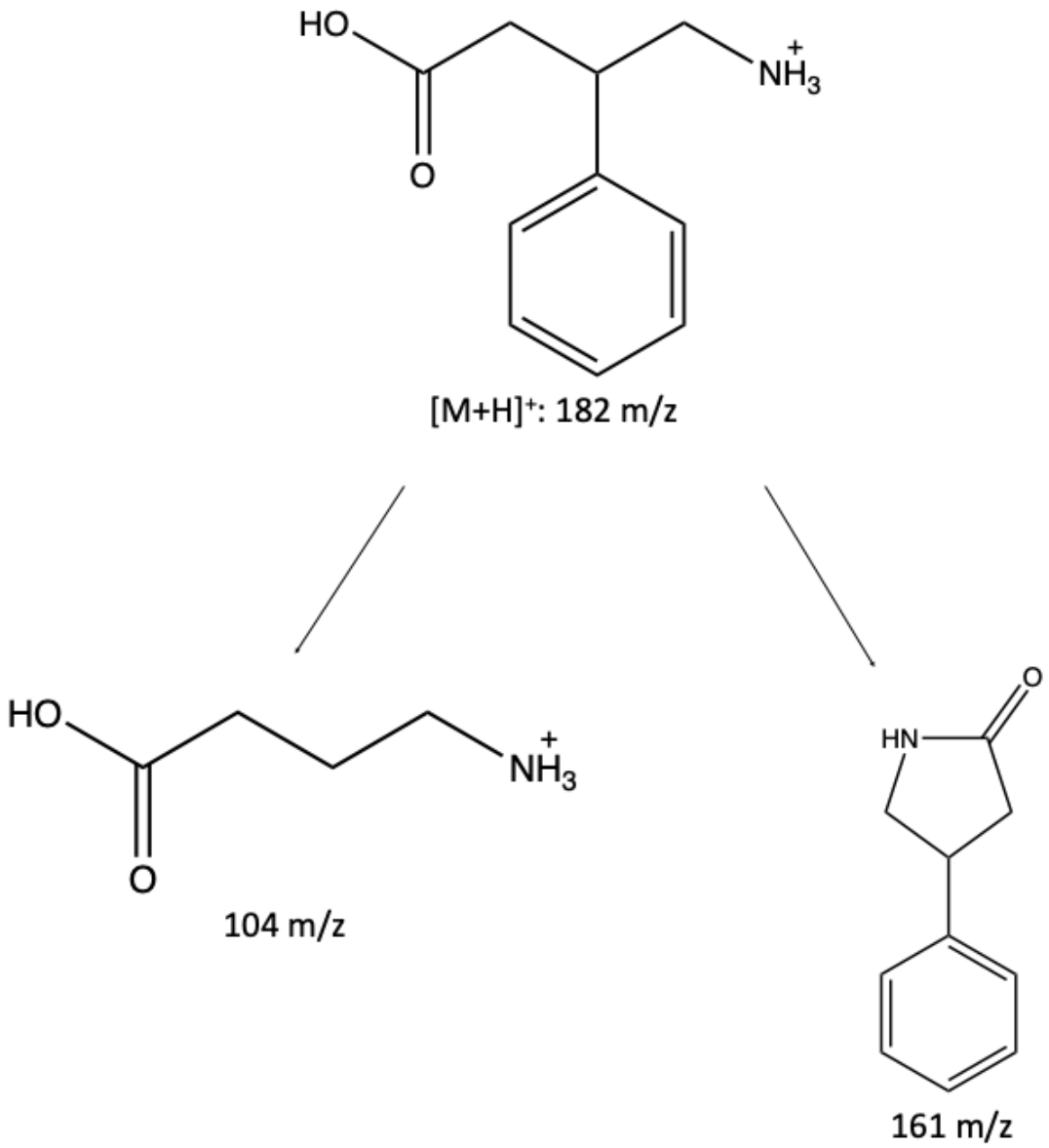

Figure 51: Fragmentation of Phenibut

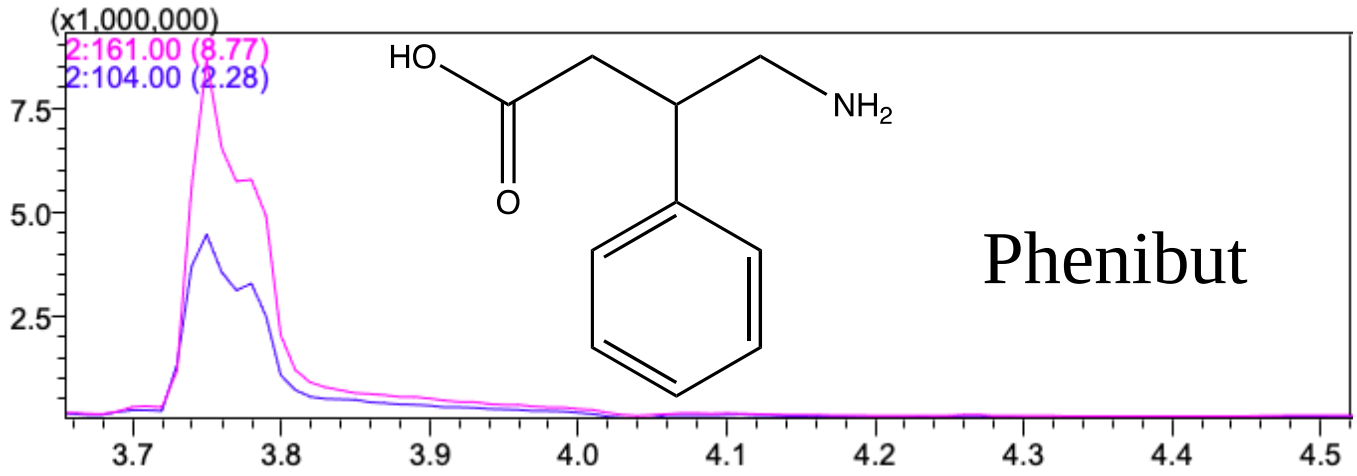

Figure 52: Moon Water Chromatogram

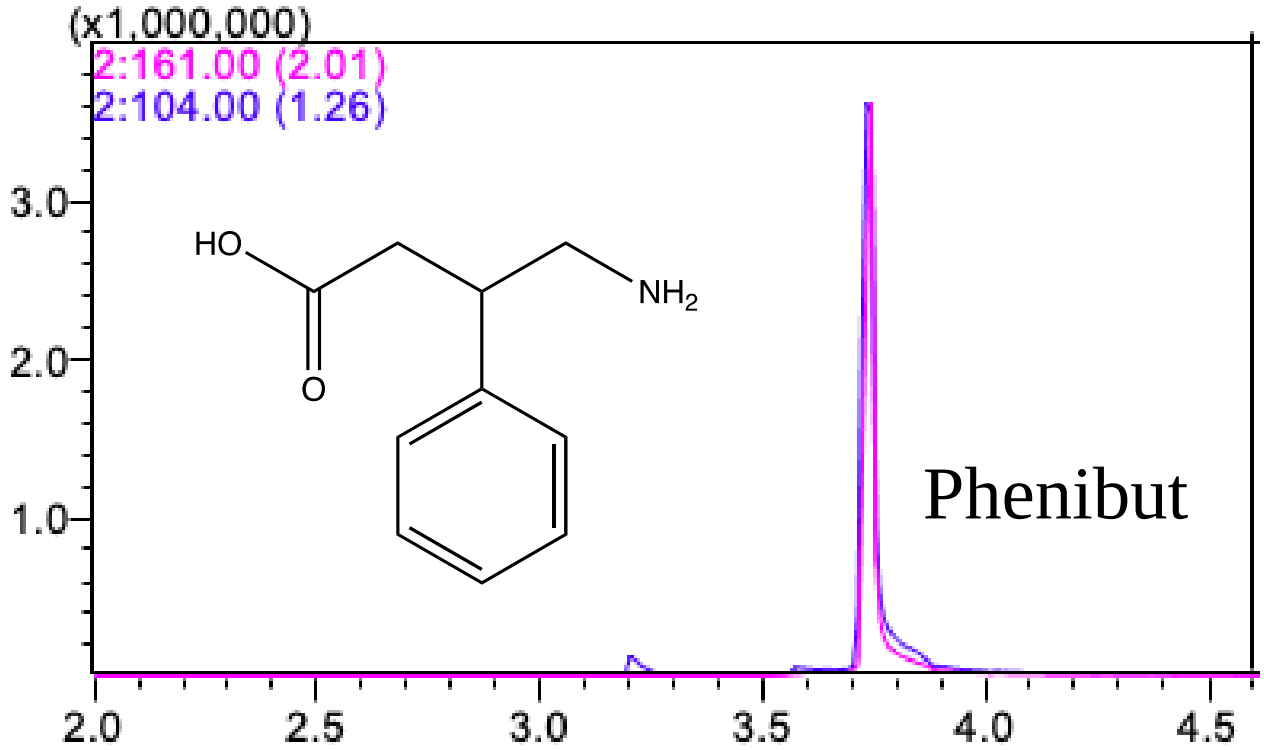

Figure 53: Phenibut Reference Standard Chromatogram

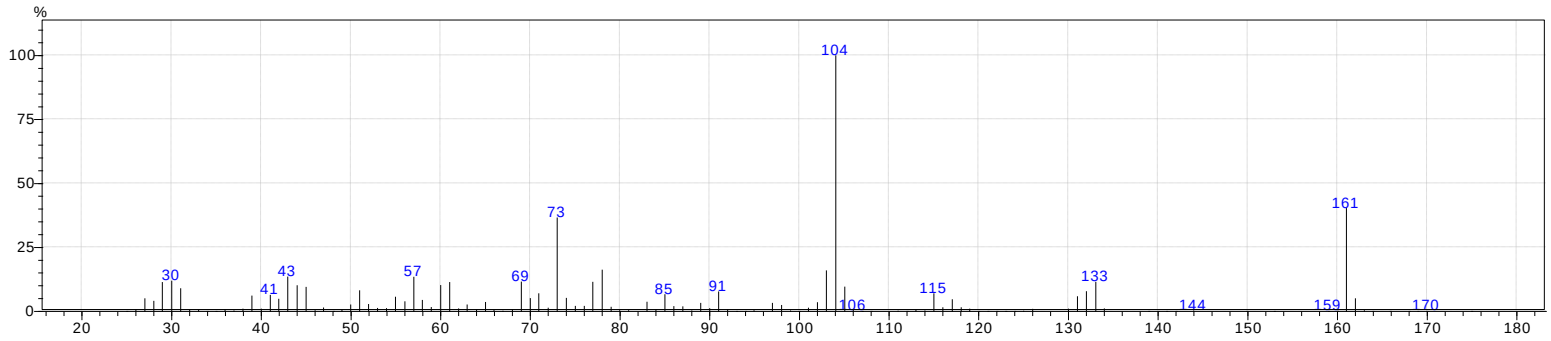

Figure 54: Moon Water Phenibut Peak Mass Spectrum

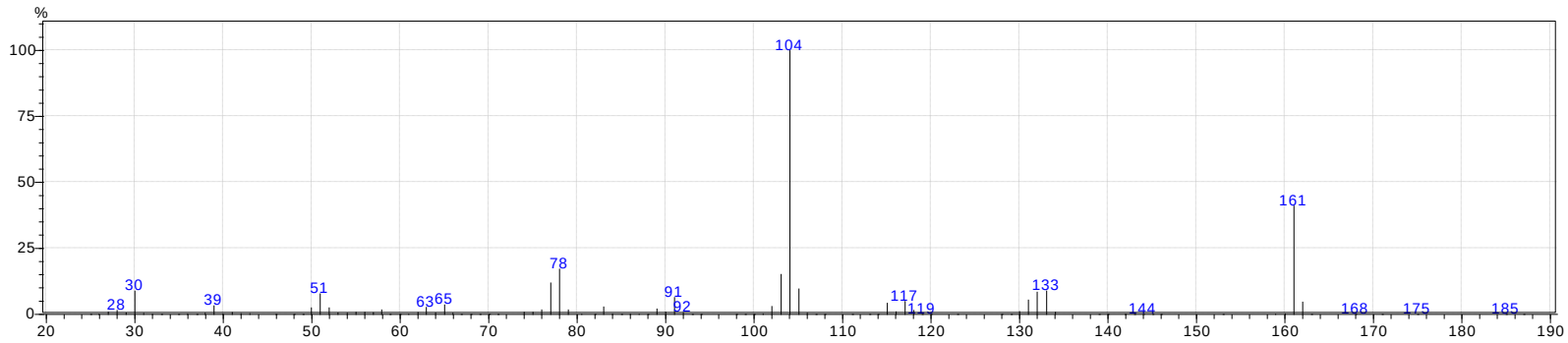

Figure 55: Phenibut Reference Standard Mass Spectrum

Vita

Originally from Greenville, South Carolina, Hunter received his B.S. in chemistry with a concentration in forensic science from Appalachian State University. He is currently a second-year Master's in Forensic Science student at Virginia Commonwealth University where he specializes in Forensic Chemistry/Drug Analysis & Toxicology. At VCU, he is involved with campus activities as the Forensic Science Graduate Organization vice president and is passionate about teaching middle/high school students about forensic science. Outside of VCU, he is an active student member of the Society of Forensic Toxicologists and American Academy of Forensic Sciences. Hunter is currently serving as a member of the AAFS Diversity Outreach Committee. When he's not in the lab, Hunter enjoys going to trivia, hiking, singing karaoke, and finding a new coffee shop to visit.