



A validated method for the quantification of mitragynine in sixteen commercially available Kratom (*Mitragyna speciosa*) products

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ABSTRACT

The recent rise in the recreational use of plant-based “legal highs” has prompted the development of methods for the identification of the bulk material, and quantification of their psychoactive components. One of these plants is *Mitragyna speciosa*, commonly referred to as Kratom. While traditional use of this plant was primarily for medicinal purposes, there has been a rise in its recreational use, and as a self-prescribed medication for opioid withdrawal. Although Kratom contains many alkaloids, mitragynine and 7-hydroxymitragynine are unique psychoactive biomarkers of the species, and are responsible for its psychoactive effects. A rapid validated method for the quantification of mitragynine in Kratom plant materials by direct analysis in real time-high-resolution mass spectrometry (DART-HRMS) is presented. It has a linear range of 5–100 $\mu\text{g mL}^{-1}$, and a lower limit of quantification of 5 $\mu\text{g mL}^{-1}$. The protocol was applied to determination of the mitragynine content of 16 commercially available Kratom plant products purchased online. The mitragynine amounts in these materials ranged from 2.76 to 20.05 mg g^{-1} of dried plant material. The utilization of DART-HRMS affords a mechanism not only for the preliminary identification of bulk plant material as being *M. speciosa*-derived (with no sample preparation required), but also provides the opportunity to quantify its psychoactive components using the same technique.

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1. Introduction

In recent years, the prevalence and use of plant-based “legal highs” has increased dramatically as users try to circumvent prosecution for the possession of illicit substances. One of these psychoactive plant drugs, *Mitragyna speciosa* (Korth.) Havil. (aka Kratom), has been heavily featured in print and news media as its recreational use becomes more popular. Kratom is a leafy tree indigenous to Southeast Asia, where it has been used traditionally for the treatment of a wide range of illnesses and ailments including fever, malaria, and chronic pain, among many others [1–3]. It is typically consumed as a powder, capsule or extract, and the leaves can also be brewed to make a tea [2,4–6]. The plant produces stimulant effects at low doses and sedative effects at higher concentrations [4,5,7–10]. Because of its dose-dependent stimulant and sedative effects, some users have reported self-medicating with Kratom for the treatment of opioid addiction, or use as a cheaper alternative to other opioids [3,6,7,11–13]. Some of the adverse side effects of Kratom include nausea, itching, sweating, vomiting, tachycardia, insomnia, and

hallucinations [3,12,14]. While ingestion of Kratom has been implicated in several cases of fatal drug overdoses [based on the presence of one of its most prominent biomarkers (mitragynine) in human blood and urine [15–19]], it could not be confirmed to be the cause of death because of: (1) the paucity of information on what constitutes a lethal dose; and (2) the fact that other drugs of abuse were also observed in these cases.

Recently, Kratom material has been introduced into the United States (U.S.) where it is easily available for purchase through the internet [1]. With the increase in its recreational use and more cases of overdoses and abuse [12,15–17,19], scrutiny of the distribution and possession of Kratom has increased. In the U.S., the Food and Drug Administration (FDA) and Drug Enforcement Administration (DEA) have both sought to regulate these products. Although the DEA listed Kratom as a Schedule I drug under the Controlled Substances Act [20], this action was reversed [21] following swift negative responses from citizens and scientists alike, who believe the plant could be used to treat opioid withdrawal and as a safer alternative to other opiates [7,11,13]. The DEA subsequently placed it on a list of “Drugs and Chemicals of Concern” along with other psychoactive plants including *Salvia divinorum* [14]. Although not federally regulated, several U.S. states have banned the possession and sale of Kratom and its products. However, as with many plant-derived materials, it is very difficult

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to visually identify plant material as being Kratom-derived when it is crushed or ground into a powder. Although individuals with training in botany are able to identify plant leaves based on their morphology, access to this type of expertise is uncommon in forensics laboratories, and most commercially available plant materials sold for recreational use are powders. Thus, it would be incredibly useful to be able to quickly identify unknown plant material, including Kratom, through its chemical profile, and quantify analytes of interest within it.

One way to identify Kratom materials is through the detection of its unique alkaloids. Although Kratom contains a large number of alkaloids (up to 37 reported by Brown et al.) [1], two in particular, mitragynine and 7-hydroxymitragynine, are species-specific biomarkers, and it is these compounds that are believed to produce its analgesic, psychoactive and stimulant effects [1,7]. While mitragynine is the most abundant alkaloid in Kratom [10], 7-hydroxymitragynine, which accounts for approximately 2% of the total alkaloid content [10], is thought to have the most potent



Fig. 1. Images of the 16 Kratom products analyzed in this study.

activity [7,22]. Mitragynine in Kratom plant material has been detected using a number of chromatographic and mass spectrometric techniques, including liquid chromatography–mass spectrometry (LC–MS) [23–25], ion mobility mass spectrometry (IMS) [23], high-performance liquid chromatography (HPLC) [26–30], gas chromatography–mass spectrometry (GC–MS) [7], and direct analysis in real time-high-resolution mass spectrometry (DART–HRMS) [31]. While it is essential that methods be available for the identification of the plant material, it is also critical to be able to quantify the mitragynine content within the products. If Kratom plant materials and commercial products become regulated, the ability to quantify mitragynine and 7-hydroxymitragynine would be essential. The quantification of mitragynine in raw plant material and other products by a number of techniques has been reported [25,26,28,29,32] with observed amounts ranging from 0.64 to 56.26 mg g^{−1} [7,28]. Plant-based beverages containing Kratom have been reported to have concentrations of mitragynine in the range of 90.021–444.23 mg L^{−1} [26,29].

As concerns over the increasing use of Kratom rise, it would be beneficial for laboratories to have a rapid means for identifying the bulk material and quantifying the analytes in plant product unknowns with the use of a single instrument. DART–HRMS, an ambient ionization mass spectrometric technique, provides a rapid approach for the mass spectral analysis of complex matrices [33–38], including the ability to detect psychoactive components in mixtures of multiple plants and/or drugs [33,39,40]. It has been used for the identification of Kratom plant materials (through detection of mitragynine and 7-hydroxymitragynine), and in distinguishing them from other plant-based legal highs, with little to no sample preparation required [31]. The rapid analysis time and limited sample preparation make DART–HRMS a highly valuable mass spectrometric tool for the identification and quantification of psychoactive plant-based “legal highs” [33–38]. DART–HRMS has been previously utilized for the quantification of analytes in other complex matrices including chlorogenic acid in *Flos Loniceræ*, [41] alkaloids in cigarette smoke [42], cholesterol content in egg pasta [43], mycotoxins in wheat and maize [44], and phosphoric esters in aqueous samples [45]. However, few reports of validated DART–HRMS methods have been described [46–48]. Those that have appeared include the quantification of atropine in *Datura* spp. seeds [46], glucocorticoids in essential oils [47], and dicyandiamide in milk [48].

Here, we present a validated method for the quantification of mitragynine by DART–HRMS, and its application for the

determination of the mitragynine content in a number of commercially available Kratom products. Recommendations from the U.S. FDA’s Bioanalytical Method Validation guidelines were used to develop the method [49]. A series of 9 standard solutions were prepared and analyzed in duplicate over three days to construct calibration curves. Four levels of quality control (QC) solutions were prepared fresh (in duplicate) for each analysis and were analyzed five times each. The resulting validated standard curves were used to determine the mitragynine content in 16 different Kratom plant products purchased from online vendors.

2. Materials and methods

2.1. Plant materials

Sixteen different Kratom plant products (Fig. 1), including a fresh plant, dried plant materials and powders, were purchased from multiple online vendors. Leaves were removed from a live Kratom plant (World Seed Supply, Medford, NY, USA) and dried at 37 °C for two days. The water content was determined to be 74%. The dried leaves were crushed using a mortar and pestle prior to analysis to form “Sample 1”. The products purchased from The Kratom King were: Maeng Da Leaf dried plant material (Sample 2); Bali/PC Leaf dried plant material (Sample 3); Bali/PC powder (Sample 4); Green Malay powder (Sample 5); Green Vein Thai powder (Sample 6); Premium Maeng Da powder (Sample 7); Red Vein Sumatra powder (Sample 8); and Green Vein Borneo powder (Sample 9). Four Kratom plant materials were purchased from Authentic Kratom: Horned Leaf Maeng Da powder (Sample 10); Maeng Da Red powder (Sample 11); Borneo Yellow Vein powder (Sample 12); and Premium Bali White Vein powder (Sample 13). Maeng Da powder (Sample 14) was purchased from Herbal Flame. Kratom plant powder in capsules (marketed as dietary supplements) was purchased from Kratom Crazy: Red Bali capsules (Sample 15); and Maeng Da capsules (Sample 16). The powdered plant materials (Samples 4–16) were too finely ground for the determination of any identifying morphological features. Photographs of the 16 Kratom products, indicated by their sample numbers, are shown in Fig. 1.

2.2. Chemical standards

Solid mitragynine (5 mg), mitragynine certified reference material (1 mg mL^{−1} in methanol) and mitragynine-*d*₃ certified

Table 1
The 16 plant products and the corresponding stock solutions made from them.^a

Sample number	Plant material	Mass of plant material (g)	Volume of stock used (μL) ^b	Volume of methanol (μL)
1	Dried leaves from fresh plant	1.0858	62.5	125
2	The Kratom King–Maeng Da Leaf	1.0590	15	172.5
3	The Kratom King–Bali/PC Leaf	1.0599	15	172.5
4	The Kratom King–Bali/PC Powder	1.0802	15	172.5
5	The Kratom King–Green Malay Powder	1.0272	15	172.5
6	The Kratom King–Green Vein Thai Powder	1.0963	15	172.5
7	The Kratom King–Premium Maeng Da Powder	1.0364	15	172.5
8	The Kratom King–Red Vein Sumatra Powder	1.0854	62.5	125
9	The Kratom King–Green Vein Borneo Powder	1.0832	15	172.5
10	Authentic Kratom–Horned Leaf Maeng Da Powder	1.0099	15	172.5
11	Authentic Kratom–Maeng Da Red Powder	1.0292	15	172.5
12	Authentic Kratom–Borneo Yellow Vein Powder	1.0257	5	182.5
13	Authentic Kratom–Premium Bali White Vein Powder	1.0446	15	172.5
14	Herbal Flame–Maeng Da Sample Powder	1.0606	15	172.5
15	Kratom Crazy–Red Bali Capsules	1.1538 ^c	5	182.5
16	Kratom Crazy–Maeng Da Capsules	0.9553 ^c	15	172.5

^a A volume of 62.5 μL of 100 μg mL^{−1} mitragynine-*d*₃ stock solution was added to each plant extract for an internal standard concentration of 25 μg mL^{−1}.

^b Different volumes of the stock extracts were used in order to accommodate the resulting points on the standard curves.

^c This mass of plant material was acquired from two (2) 500 mg capsules.

reference material ($100 \mu\text{g mL}^{-1}$ in methanol) chemical standards were purchased from Cayman Chemical (Ann Arbor, MI, USA). HPLC grade methanol solvent was purchased from Pharmco Aaper (Brookfield, CT, USA). High-purity helium was purchased from Airgas (Albany, NY, USA).

2.3. Preparation of standard solutions

A master stock solution of mitragynine in methanol ($8900 \mu\text{g mL}^{-1}$) was prepared. Serial dilutions were performed to create solutions with mitragynine concentrations of 200, 150, 100, 80, 50, 20, and $10 \mu\text{g mL}^{-1}$. These solutions were then doped with mitragynine- d_3 , the internal standard. The final concentrations of mitragynine in the 7 non-zero calibrator solutions were 100, 75, 50, 40, 25, 10, and $5 \mu\text{g mL}^{-1}$ with an internal standard concentration of $25 \mu\text{g mL}^{-1}$.

2.4. Preparation of quality control (QC) solutions

The QC solutions were created from the 1 mg mL^{-1} mitragynine in methanol certified reference material stock solutions. Two solutions were made for each concentration level. The final mitragynine concentrations of the QCs were 90, 60, 15, and $5 \mu\text{g mL}^{-1}$ with an internal standard concentration of $25 \mu\text{g mL}^{-1}$. For each of the three accuracy and precision analytical runs, fresh QCs were created (as specified by FDA guidelines).

2.5. Preparation of plant extracts

Approximately one gram of each plant material was added to 10 mL of methanol and the suspensions were allowed to stand overnight. A portion (2 mL) of the supernatant was removed and used to prepare stock solutions. Different volumes of each plant

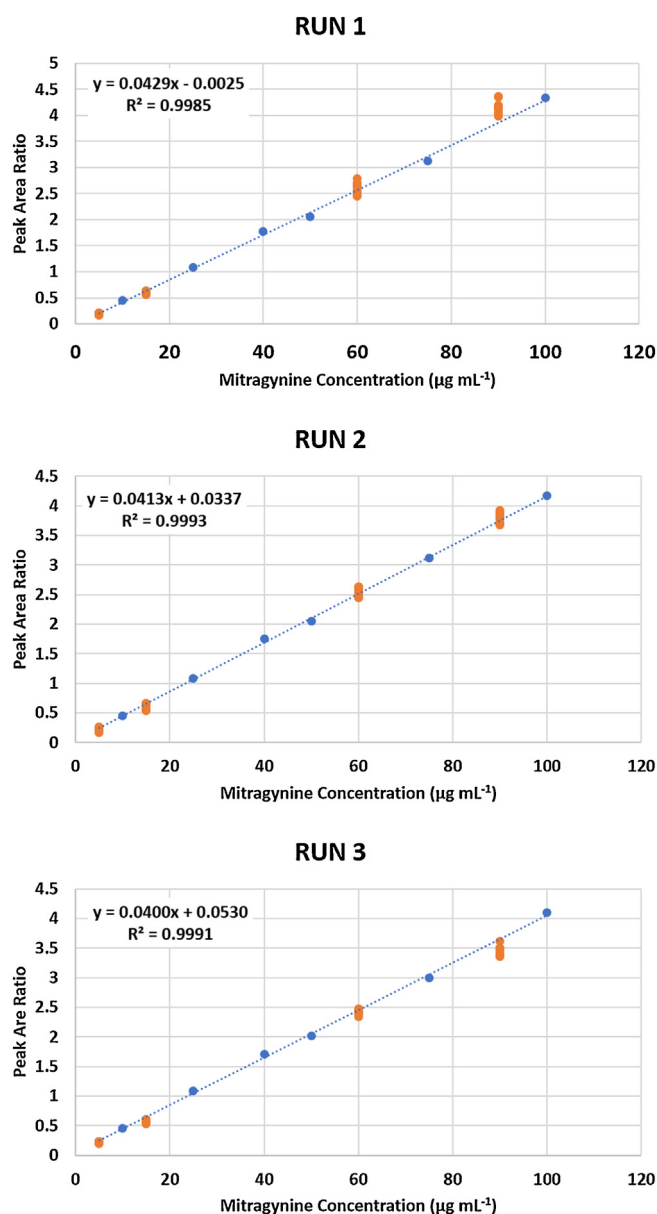


Fig. 2. The validated standard curves for the three accuracy and precision analytical runs. The x-axes correspond to the mitragynine concentration ($\mu\text{g mL}^{-1}$) and the y-axes are the calculated peak area ratios (PAR). The R^2 values are all above 0.998 and are displayed on the curves. The slopes of the regression lines were determined to be statistically identical by an analysis of covariance (ANCOVA). (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

extract (Table 1) were diluted with methanol to a final volume of 187.5 μL . To this, 62.5 μL of the 100 $\mu\text{g mL}^{-1}$ mitragynine- d_3 stock solution was added, for a final internal standard concentration of 25 $\mu\text{g mL}^{-1}$.

2.6. Direct analysis in real time-high-resolution mass spectrometric (DART-HRMS) analysis

All analyses were completed using a DART-SVPTM ion source (IonSense, Saugus, MA) coupled to a JEOL AccuTOF high-resolution mass spectrometer (JEOL USA, Inc., Peabody, MA). The mass spectrometer utilized has a resolving power of 6000 (FWHM definition) and was operated with an orifice 1 voltage of 20 V and orifice 2 and ring lens voltages of 5 V each. The ion guide voltage was set to 600 V to allow for the detection of ions above m/z 60. The ion source was operated in positive ion mode with a helium gas flow rate of 2 L min^{-1} and a gas heater temperature of 350 $^{\circ}\text{C}$. An automated linear rail system (IonSense, Saugus, MA) was used to suspend 12 DipItTM tips (IonSense, Saugus, MA) at a time, and move them automatically in front of the ion source at a rate of 1 mm s^{-1} . DipItTM tips (glass capillaries) were dipped into the Eppendorf tubes (0.6 mL) containing each calibrator solution before being suspended on the linear rail system. Calibrator and QC solutions were analyzed two and five times, respectively, from the lowest to highest concentration of mitragynine.

2.7. Validation of the calibration curve

For the validation of the quantification method, the FDA's recommendations for a bioanalytical method [49] were followed. The guidelines require that three accuracy and precision analyses be completed over several days for the validation of the quantification method's standard curve. The calibrator series of solutions must contain a blank (no mitragynine or internal standard), a zero calibrator (blank with internal standard), and at least six non-zero calibrator solutions. Two solutions of QCs at four concentrations including the lower limit of quantification (LLOQ), low- (defined as 3 \times LLOQ), mid- (defined as mid-range) and high-range (defined as high-range) were analyzed with five replicates each in at least three runs (one analysis per day). To accept the accuracy and precision runs for validation, the following conditions had to be reached within and between the three separate analyses: (1) the analyte response at the LLOQ should be 5 \times the zero calibrator; (2) the accuracy should be $\pm 15\%$ of the nominal concentration of non-zero calibrators and $\pm 20\%$ for the LLOQ; and (3) the non-zero calibrators' coefficients of variation value should be $\pm 15\%$, except for $\pm 20\%$ at the LLOQ. These parameters must also be met for each QC solution. For both

requirements (2) and (3), 75% or more of the non-zero calibrators should meet the listed criteria.

2.8. Determination of mitragynine concentrations in plant extracts

The plant samples were tested in three separate analyses with five replicates each. The plant-based solutions were created such that the levels of analytes of interest could be accommodated on the calibration curve. The equation for the standard curve line was used to calculate the concentrations of mitragynine in each plant sample.

3. Results and discussion

3.1. Validation of the mitragynine calibration curve

To achieve the requirements for a validated method as defined by the U.S. FDA, a series of solutions ranging from 5 to 100 $\mu\text{g mL}^{-1}$ mitragynine in methanol were used as the non-zero calibrators. The extracted ion chromatograms for m/z 399.2284 and m/z 402.2472, corresponding to protonated monoisotopic mitragynine and mitragynine- d_3 respectively, were used for peak integration. The areas under the mitragynine peaks were divided by the areas under the mitragynine- d_3 peaks to calculate the peak area ratio (PAR). The PARs were plotted against the concentration of mitragynine ($\mu\text{g mL}^{-1}$) for the determination of the standard curve. The PARs from which the standard curves were derived, for each of the three accuracy and precision analytical runs, are shown in Supplementary Tables 1, 2 and 3, respectively.

As an ambient ionization technique, DART-HRMS can be affected by environmental factors, such as humidity and air flow disturbances at the open-air sampling gap between the ion source and MS inlet, and these can impact the signal intensities of analytes of interest between replicates [50]. In order to address these potential issues, an internal standard was used. By calculating the PAR for each replicate, the between sample signal variation was minimized because the ratio between the analyte of interest and the internal standard remained the same regardless of the absolute counts or areas under the curve. Another potential cause for concern with utilizing DART-HRMS for quantification purposes, is its mechanism of ionization which relies heavily upon the proton affinity of the ions of interest. If the chosen internal standard is not efficiently ionized to the same extent as the analyte of interest, the PARs may not respond linearly and would not be effective for the determination of the standard curve. This was addressed by using the isotopically labelled internal standard mitragynine- d_3 . This ensured that the ionization efficiency of both the analyte of interest and the internal standard were identical.

Table 2

The within-run and between-run means, relative errors, and coefficients of variation for the four QC solutions.

QC concentration ($\mu\text{g mL}^{-1}$)	Within-run			Between-run		
	Mean	Relative error (%)	Coefficient of variation (%)	Mean	Relative error (%)	Coefficient of variation (%)
5	4.51	−9.71	7.73	4.28	−14.34	10.89
	4.19	−16.29	14.93			
	4.15	−16.99	5.90			
15	13.87	−7.54	4.26	13.59	−9.41	6.64
	13.76	−8.23	8.56			
	13.13	−12.46	4.75			
60	60.81	1.35	4.31	60.21	0.35	3.44
	60.85	1.42	2.80			
	58.98	−1.70	1.53			
90	97.36	8.18	2.94	91.13	1.26	6.14
	91.22	1.35	2.08			
	84.81	−5.76	2.17			

Fig. 2 shows the standard curves derived from the three separate accuracy and precision analytical runs that were completed over several days. The blue dots are representative of the non-zero calibrator solutions (the same solutions were used for all three runs) and the orange dots correspond to the four QCs (new solutions were used for all three runs). The LLOQ was determined to be $5 \mu\text{g mL}^{-1}$ mitragynine in methanol. The R^2 values for the three analytical runs were all above 0.998 and the between-run and within-run means, relative errors, and coefficients of variation for each set of QCs (LLOQ, low-, mid-, and high-range) are listed in Table 2.

The relative errors and coefficients of variation all fell within the required parameters for a validated method. Thus, the calibrator solutions and standard curves produced could be used for the quantification of mitragynine in the 16 plant materials. As shown in Table 2, the within-run means decrease with each analytical run. While this could suggest that the solutions are degrading over time, all three analytical runs met the requirements set by the FDA for a validated method and thus were used for the quantification of mitragynine in plant materials. Since the QC solutions are made fresh for each analysis, there is a built-in system for determining when the solutions have become too degraded for the calculation of mitragynine in plant materials.

3.2. Determination of mitragynine concentrations in plant material

Prior to the quantification of mitragynine in plant material, a number of solvents were tested for their ability to extract mitragynine derivatives from Kratom. The methanolic extract resulted in the highest absolute counts for mitragynine and 7-hydroxymitragynine, and thus methanol was chosen as the extraction solvent. Additionally, before the quantification of mitragynine was performed, the methods of extraction were analyzed to confirm the efficient extraction of the analytes of interest. Extracts were prepared for Samples 2 and 3 before and after grinding of the plant material to test for extraction efficiency. The absolute counts of mitragynine and 7-hydroxymitragynine in both extracts were determined to be statistically identical by an analysis of variance (ANOVA). Therefore, to reduce sample preparation steps these plant materials were extracted as is, without additional grinding prior to solvent exposure. Fig. 3 shows a representative mass spectrum of one of the plant material extracts (Sample 2). In the mass spectrum, the peak corresponding to protonated mitragynine (m/z 399.2263) dominated the spectrum. The peak at m/z 415.2175 is representative of protonated 7-hydroxymitragynine. A similar pattern was observed for all 16 plant materials.

For the quantification experiments, the plant material extracts were analyzed with five replicates each. The extracts were tested with the same set of standard calibrator solutions used in the validated curves. The PARs of the standard solutions were used to create a standard curve for each of the three analyses for the quantification of mitragynine in plant materials. The calculated average concentrations of each plant material extract (as shown in Table 1) are listed in Table 3 with their corresponding standard deviations and coefficients of variation. The plant extracts were made from different amounts of plant material and volumes taken from each stock solution as indicated in Table 1. The last column of Table 3 lists the observed amounts of mitragynine per g of dried plant material.

The mitragynine content found in these plant materials is consistent with those previously reported [7,25,26,28,29]. The dried leaves from the live plant (Sample 1) had the least amount of mitragynine per g of dried plant material, followed closely by Sample 8, a powdered plant material labelled as “Red Vein Sumatra”. The commercially available dried plant products (Samples 2 and 3) appeared to contain more than just leaf material and may have included stems and bark. However, it is unknown what parts of the Kratom plant were used in the powdered products. Samples 15 and 16 were products comprised of powdered plant material placed within capsules, and were found to contain the highest concentrations of mitragynine per g of material. According to Charoonratana et al. [51], the mitragynine content in the leaves of Kratom plants is higher than in the stems and bark. As is common with plant-derived products, the age of the plant, climate conditions and growth locale can also have an effect on the chemical composition of their leaves. For example, Takayama reported differences in mitragynine content from Kratom plants acquired from Thailand and Malaysia [10].

While the high mitragynine levels in the capsules may have been an inherent attribute of the plants from which it was derived, it is also possible that the product was spiked with additional mitragynine for greater effect. Lydecker et al. reported the suspected addition of 7-hydroxymitragynine in Kratom products purchased online [32]. 7-Hydroxymitragynine has been found to account for only ~2% of the total psychoalkaloid content. However, the amounts can vary between different plants and also within the same plant for differently sized leaves [24]. For this reason, the ratio of mitragynine to 7-hydroxymitragynine in and of itself, cannot yet be used to infer that a plant product has been spiked. Both molecules must be quantified independently, and the approach reported here can be used not only for mitragynine, but also for 7-hydroxymitragynine, and any other component of interest.

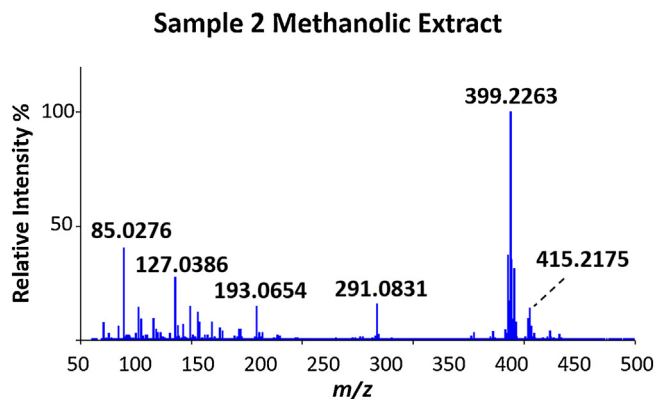


Fig. 3. A representative DART-HRMS mass spectrum of the methanolic extract of Sample 2. The protonated peaks for mitragynine and 7-hydroxymitragynine are m/z 399.2263 and 415.2175, respectively.

Table 3

The average mitragynine concentration in each of the 16 plant materials.

Sample number	Average mitragynine concentration ($\mu\text{g mL}^{-1}$)	Standard deviation	Coefficient of variation (%)	Mitragynine content (mg g^{-1})
1	74.95	2.75	3.66	2.76
2	89.11	1.44	1.61	14.02
3	68.16	0.94	1.38	10.72
4	88.20	1.40	1.59	13.61
5	80.16	0.99	1.24	13.01
6	76.33	1.99	2.61	11.60
7	69.84	0.70	1.01	11.23
8	85.82	2.39	2.78	3.16
9	88.14	1.62	1.83	13.56
10	75.32	2.75	3.65	12.43
11	57.24	1.97	3.44	9.27
12	22.33	0.60	2.67	10.88
13	75.29	3.97	5.28	12.01
14	86.91	3.40	3.91	13.66
15	46.27	1.97	4.26	20.05
16	95.45	6.06	6.35	16.65

Although the mitragynine content in Kratom-derived products has been quantified by other methods, the DART-HRMS approach has several advantages. Before the psychoactive components of plant materials can be quantified, the plant material must first be identified. Unlike cannabis, most other psychoactive plants are not easily visually identifiable, especially in the dried and ground forms available for purchase in the U.S. By the most commonly used conventional methods (GC–MS and LC–MS) the identification process requires several pretreatment steps, including extractions and sample clean-up, prior to the analysis [25,29]. In contrast, DART-HRMS can be used to quickly identify bulk plant material by direct analysis with no sample pre-treatment [31,33–35,37,38], and can also be used, in the same experimental analysis, to detect and identify adulterants or diluents, using their high-resolution masses, and isotope ratio matching [36]. After the rapid presumptive identification of Kratom, the same technique can then be used to quantify the major alkaloid mitragynine or 7-hydroxymitragynine through analysis of an easily prepared methanolic extract. The utility of DART-HRMS as a preliminary screening tool, followed by its use for the quantification of the analyte of interest, provides a means for the increasing number of laboratories in the U.S. with DART-MS equipment, to use a single instrument for identification and quantification of compounds in a high-throughput manner, and can assist in the reduction of casework backlogs. The DART-HRMS analysis of both blanks (in duplicate), 7 non-zero calibrator solutions (in duplicate), 4 QC solutions (five replicates each), and 16 plant samples (five replicates each) was accomplished within 30 min. As the automated linear rail system was utilized, this technique was semi-automated, minimizing the human error that can occur with manual sampling.

4. Conclusions

A validated DART-HRMS method for the quantification of mitragynine is described. The linear range of quantification was 5–100 $\mu\text{g mL}^{-1}$ mitragynine in methanol with a LLOQ of 5 $\mu\text{g mL}^{-1}$. The developed method was subsequently used for the determination of the mitragynine content in 16 commercially available plant samples, including fresh and dried plant material, powdered plant material, and capsules sold as dietary supplements. The lowest concentration was found in the dried leaves of the live plant and the highest in the capsules, with a range of 2.76–20.05 mg g^{-1} . The speed of analysis and limited sample preparation are advantages of the utilization of the DART-HRMS technique.

CRediT authorship contribution statement

Kristen L. Fowble: Methodology, Validation, Investigation, Writing - original draft, Visualization. **Rabi A. Musah:** Conceptualization, Methodology, Data curation, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.forsciint.2019.04.009>.

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