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Development and Validation of a Method for Detecting and Quantifying Mitragynine in Kratom Samples Using HPLC-PDA

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ABSTRACT / ABSTRAK

Kratom (Mitragyna speciosa Korth) has been identified as a New Psychoactive Substance (NPS) by the United Nations Office on Drugs and Crime (UNODC) and included in the list of prohibited ingredients in food supplements and traditional medicine by Indonesian FDA. Therefore, a rapid, easy, and reliable analytical method is necessary to detect and quantify this plant and its products. This study developed a method for the detection and quantification of kratom products based on a unique compound, mitragynine, as a biomarker. A previous survey of determining mitragynine in Kratom using GC-MS, LC-MS/MS, UPLC, and HPLC-PDA. Previously, the HPLC-PDA method used a C8 column. Yet, for efficiency, it is also necessary to develop a test method using a C18 column. Analysis was performed using an HPLC - PDA system with Waters Atlantis T3-C18 (250 x 4.6 mm, 5 µm) column. The mobile phase comprises acetonitrile and formic acid 0.05%, pH 5.0 (75:25 v/v), delivered at a 1.0 mL/min flow rate. Detection was carried out at a wavelength of 225 nm. The analytical method was validated with test parameters of selectivity, system suitability, accuracy, precision, linearity, detection limit, and quantification limit. The validation study demonstrated an excellent linear concentration range of 1.96 - 6.01 µg/mL with a correlation coefficient of 0.9996; the detection limit is 0.14 µg/mL, while the limit of quantification is 0.45 µg/mL—accuracy method of 98.88 - 101.44% and a bias of 0.27%. The percent relative standard deviation for six independent assay determinations was 0.67%, and the intermediate precision was 1.56% on two days. The mitragynine amounts in these materials ranged from 6.01 to 6.31 mg/g of dried leaf material. Based on the research results, it can be concluded that the method developed provides quick, simple, reliable, accurate, and valid, and has an advantage over existing methods in terms of simplicity of sample preparation, short analysis time, and cost-effectiveness compared to GCMS and LCMS/MS and can be applied for future analysis in Kratom samples.

Kratom (Mitragyna speciosa Korth) telah ditetapkan sebagai senyawa psikoaktif baru (NPS) oleh United Nations Office on Drugs and Crime (UNODC) dan digolongkan ke dalam daftar bahan terlarang dalam suplemen makanan dan obat tradisional oleh BPOM. Oleh karena itu, diperlukan metode analisis yang cepat, mudah, dan andal untuk mendeteksi dan menetapkan kandungan tanaman tersebut dan produk turunannya. Pada penelitian ini dikembangkan metode untuk mendeteksi dan menetapkan kadar produk kratom berdasarkan senyawa unik, mitragynine sebagai biomarker. Penelitian sebelumnya yang telah dikembangkan menggunakan GC-MS, LC-MS/MS, UPLC dan HPLC-PDA. Metode HPLC-PDA yang tersedia menggunakan kolom C8, namun untuk efisiensi, perlu juga dikembangkan metode pengujian dengan menggunakan kolom C18. Analisis dilakukan menggunakan sistem KCKT - PDA yang dilengkapi dengan kolom Waters Atlantis T3-C18 (250 x 4,6 mm, 5µm). Fase gerak terdiri dari asetonitril dan asam

format 0,05%, pH 5,0 (75:25 v/v) dengan laju alir 1,0 mL/menit. Deteksi dilakukan pada panjang gelombang 225 nm. Validasi metode ditunjukkan dengan parameter uji: selektifitas, akurasi, presisi, linieritas, batas deteksi dan batas kuantifikasi. Metode identifikasi dan penetapan kadar mitragynine secara KCKT-PDA menunjukkan hasil uji linier pada rentang konsentrasi 1,96 - 6,01 µg/mL dengan koefisien korelasi 0,9996. Batas deteksi metode yaitu 0,14 µg/mL, sedangkan batas kuantifikasi 0,45 µg/mL. Uji akurasi metode adalah 98,88 - 101,44% dan bias 0,27%. Presisi metode dan intermediet secara berturut-turut yaitu adalah 0,67% dan 1,56%. Kandungan mitragynine berkisar antara 6,01 hingga 6,31 mg/g bahan daun kering. Berdasarkan hasil penelitian, dapat disimpulkan bahwa metode yang dikembangkan cepat, sederhana, andal, akurat, dan valid serta memiliki keunggulan dibandingkan metode yang ada dalam hal kesederhanaan persiapan sampel, waktu analisis yang singkat, dan efektivitas biaya dibandingkan dengan GCMS dan LCMS/MS dan dapat diaplikasikan untuk analisis sampel Kratom.

Keywords: Kratom, Mitragynine, Mitragyna speciosa, Detection, Quantification, HPLC-PDA
Kata kunci: Kratom, Mitragynine, Mitragyna speciosa, deteksi, kuantifikasi, KCKT-PDA

1. Introduction

Kratom (*Mitragyna speciosa* Korth) is a plant of the Rubiaceae family from Southeast Asia (Muang Thai, Indonesia, Malaysia, Myanmar, Philippines) and Papua New Guinea. It is a Putussibau, Kapuas Hulu, and West Kalimantan herb characteristic. The leaves are most widely used in Kratom, and it is consumed by chewing, smoking, and boiling like tea. (Sanagi et al., 2013).

Figure 1. Kratom plant (a). Kratom leaves, (b) and (c). Kratom tree

Kratom has been identified as a New Psychoactive Substance (NPS) by the United Nations Office on Drugs and Crime (UNODC) in the World Drug Report since 2013 (UNODC, 2013). Accordingly, the Indonesian Food and Drug Authority (FDA) issued an Announcement Letter Number. HK. 04.4.42.421.09.16.1740 concerning the Prohibition of Mitragyna speciosa (Kratom) in Traditional Medicines and Health Supplements. This is according to the Decree of the Head of the Indonesian FDA Number HK.00.05.23.3644 Appendix 3 concerning Basic Provisions for Supervision of Food Supplements and Regulation of the Head of Indonesian FDA Number HK.00.05.41.1384 Attachment 4, "Criteria and Procedure for Registration of Traditional Medicines, Standardized Herbal Medicines, and Phytopharmaca." It was stated that Mitragyna speciosa (Kratom) is included in the list of prohibited ingredients in food supplements and traditional medicine (BPOM, 2005a, 2005b, 2016). However, the National Narcotics Board granted a transition period

until 2024 to classify Kratom into the Class I Narcotic List published by the Ministry of Health (BNN, 2020).

Kratom can be obtained easily in online shops with low prices; therefore, Kratom and its product are abused as alternatives to other narcotics like heroin or marijuana. (Elsa, 2016). The pharmacological effects were reported as pain relievers. (Carpenter et al., 2016; Reanmongkol et al., 2007; Shamima et al., 2012), sedative (Novindriani, 2014), stimulant, antidepressant (Moklas et al., 2008), anti-inflammatory (Tohar et al., 2019), antidiarrheal (Suhaimi, S.; Kartikasari, 2020), antioxidant, and antibacterial (Parthasarathy et al., 2009). Kratom contains alkaloid indole, mitragynine, and 7-hydroxy mitragynine with antinociceptive pharmacological properties. (Shamima et al., 2012). However, some adverse effects associated with the exposure have been reported, such as psychosis, seizures, intrahepatic cholestasis, other medical problems, and fatalities. (Fluyau & Revadigar, 2017). Therefore, a rapid, easy, and reliable analytical method is necessary to detect and quantify this plant and its products.

Mitragynine (Fig. 2), a major alkaloid kratom, binds to the μ-opioid and δ-opioid receptors, while 7-hydroxy mitragynine has a stronger affinity to μ-opioid receptor than other receptors (Matsumoto, 2006). Mitragynine showed 16 times more affinity for μ-opioid and δ-opioid receptors and 200 times less affinity than morphine (Yue et al., 2018), resulting in analgesic opioid activity for both mitragynine and 7-hydroxy mitragynine (Swogger & Walsh, 2018). Mitragynine has stimulant effects at low doses and sedative effects at high doses (Swogger & Walsh, 2018), and it is not found in other plants (Kikura-Hanajiri et al., 2009). This compound is exclusively found in M. speciosa but not in any other genus of Mitragyna (Lesiak et al., 2014; Sanagi et al., 2013). Therefore, it may be used as a marker compound for identifying Kratom.

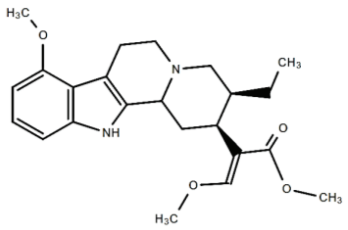


Figure 2. Structure molecule of mitragynine

Some methods for analyzing mitragynine in kratom plant material have been reported. (Elsa, 2016; Flores-Bocanegra et al., 2020; Kikura-Hanajiri et al., 2009; Lelono et al., 2021; Lesiak et al., 2014; Mudge & Brown, 2017; Parthasarathy et al., 2013). Lelono et al. Identified mitragynine in kratom leaves by TLC, GCMS, and LCMS/MS. Mudge, E. M., and Brown determined mitragynine in Mitragyna speciosa raw materials and finished products by liquid chromatography and UV detection. (Mudge & Brown, 2017). Furthermore, Casey et al. conducted a quantitative and qualitative analysis of mitragynine in Kratom (Mitragyna speciosa) by GC-MS, LC-MS/MS, and UPLC-PDA. Mitragynine in kratom products was quantitated by UPLC and LCMS/MS using a mobile gradient phase of 0.1% aqueous formic acid and acetonitrile. Parthasarathy et al. developed and validated an analytical method using an HPLC-PDA system with Inertsil C8 (150 x 4.6 mm, 5 μm) as the column and a mix of acetonitrile and formic acid, 50:50 (v/v), as the mobile phase. Analysis with the HPLC method is more straightforward, sensitive, and inexpensive than GCMS, LC-

MS/MS, and UPLC. Previous studies by HPLC have been limited to using C8 columns, so it is necessary to research different types of columns to provide alternative columns. Considering this, this study aims to develop and validate a method for identifying and quantifying the amount of mitragynine in Kratom leaves using HPLC - PDA, which is faster, more inexpensive, and more sensitive but remains accurate, specific, and selective. Different types of columns were also used to add alternative column usage. This method was validated on the test parameters of selectivity, system suitability, accuracy, precision, linearity, detection limit, quantification limit, robustness, and stability study according to ICH guidelines.

2. Methodology

2.1. Plant Material

Dried leaves of M. speciosa Korth. (Rubiaceae) were collected from Putussibau, Kapuas Hulu, West Kalimantan Province, Indonesia, in December 2020.

2.2. Chemicals

Mitragynine standard Supelco and 7-Hydroxymitragynine standard Supelco were purchased from Cerriliant Corporation (USA), reagents for analysis: methanol, chloroform, n-hexane, ethyl acetate, ammonia 25%, methanol gradient grade for LC, acetonitrile gradient grade LC, formic acid 98-100%, sodium hydroxide solution 1 N (Merck, German), water for HPLC was prepared using Milli-Q water purifier system (Merck, German), TLC, HPTLC silica gel 60 F_{254}, Silica Gel 60 (0.063 – 0.200 mm) for column chromatography (Merck, German), membrane filter PTFE 0.45 µm Agilent (USA).

2.3. Chromatographic conditions

The method was developed on a Shimadzu LC-20AD Prominence HPLC system coupled to a photodiode array detector (Shimadzu, Japan). Chromatographic separation was achieved at 40°C on a Waters Atlantis T3-C18 column (250 x 4.6 mm, 5 µm) (Waters, MA, USA). The mobile phase was acetonitrile and 0.05% formic acid (adjusted to pH 5 with NaOH 1 N), 75:25 (v/v), running in an isocratic mode at a 1.0 mL/min flow rate. The volume injection of the sample was 20 µL, and the total analytical run time was 8 min with mitragynine eluting at 6.2 min. Detection was carried out at 200 - 400 nm, while the UV signal at 225 nm was extracted for quantification. Mitragynine identification compares the retention time of the HPLC and the UV spectrum of the analyte with that of the mitragynine standard. Peak purity was obtained by PDA chromatogram.

2.4. Validation of the HPLC method

This method was validated according to ICH guidelines on the test parameters of selectivity, system suitability, accuracy, precision, linearity, detection limit, quantification limit, and robustness.

A blank solution was methanol HPLC grade. Subsequently, 100 µg/mL of mitragynine-certified standard reference solution in methanol and 100 µg/mL of 7-hydroxy mitragynine-certified standard reference solution in methanol. The specificity solution was a mixture of 7-hydroxy mitragynine and mitragynine standard in methanol (4.0 µg/mL). Working standard solutions of 2.0, 3.0, 4.0, 5.0, and 6.0 µg/mL mitragynine standard were prepared. A calibration curve was created on each day of analysis. Six replicates of mitragynine isolate were analyzed on two consecutive days to evaluate the within-day and between-day precision. Accuracy was assessed as trueness (% bias) between the content of mitragynine

standard quantified with calibration curve and at the certificate. Recovery was performed by spiking the standard to sample with a ratio (30:70) at three-level concentrations. Accuracy and recovery were calculated using Eq. (1-3) below.

$$\%\ accuracy\ (\%\ bias) = \frac{K_b - K_d}{Kb}\ x\ 100\% \quad (1)$$

$$K_d = \frac{a}{b}\ x\ 100\% \quad (2)$$

where :
$a = calculated\ concentration\ based\ on\ calibration\ curve$
$b\ = theoretical\ concentration$
$K_b = content\ at\ certificate$
$K_d = calculated\ content$

$$\%\ recovery = \frac{total\ concentration\ of\ mitragynine - \ concentration\ of\ spike\ sample}{concentration\ of\ standard}\ x\ 100\% \quad (3)$$

The robustness method was used by quantifying mitragynine at varying flow rates (0.8 mL/min, 1.0 mL/min, and 1.2 mL/min) and wavelengths (223 nm, 225 nm, and 227 nm). The data were analyzed using a single ANOVA factor.

2.5. Preparation and analysis of M. speciosa samples

The analytical method was developed and applied with authentic samples from *M. speciosa*. The dried leaves of *Mitragyna speciosa* Korth (400 g) were powdered and extracted sequentially using n-hexane, chloroform, and methanol corresponding to the Mustafa et al. method. In the current study, the ratio of solid to solvent is 1:10. For each solvent, extraction assisted ultrasonication for 10 minutes and extraction for 2 hours at room temperature with an orbital shaker. The methanol extract was concentrated with a rotary evaporator to yield the residue (67.94 g). In addition, the methanol extract (2 g) was subjected to column chromatography using silica gel 60 (0.063 - 0.200 mm) eluting with n-hexane - ethyl acetate - ammonia 25% (30:15:1) to yield fraction A-G. Fraction G was separated by HPTLC silica gel 60 F 254 (n-hexane - ethyl acetate - ammonia 25% (30:15:1)) to obtain isolate (8.10 mg).

The samples of kratom were prepared by sequential extraction according to the preceding method. First, the methanol extract (50 mg) was dissolved in 25.0 mL methanol with sonication assistance for 2 minutes. Next, 0.5 mL of the solution was diluted in 10.0 mL of methanol and filtered through a PTFE syringe filter (Agilent, USA). Finally, samples were injected into an HPLC system. Mitragynine content was quantified by HPLC-PDA based on the calibration curve of mitragynine standard and isolate. Data results were compared statistically with Anova's single factor.

2.6. Stability of mitragynine

The short-term stability of mitragynine at a concentration of 4 µg/mL in methanol was evaluated at room temperature (24 - 26°C) during 0 - 72 hours.

3. Result and Discussion

3.1. Optimisation of the chromatographic conditions

Some methods for analyzing mitragynine in kratom plant material using TLC, GCMS, LCMS/MS, HPLC, and UPLC have been reported. (Elsa, 2016; Flores-Bocanegra et al., 2020; Kikura-Hanajiri et al., 2009; Lelono et al., 2021; Lesiak et al., 2014; Mudge & Brown, 2017; Parthasarathy et al., 2013). Analysis with the HPLC method is a simple, fast, easy-to-use, selective, accurate, and cost-effective method for testing Mitragynine. (Janchawee et al., 2007; Mudge & Brown, 2017; Parthasarathy et al., 2013)Parthasarathy et al. developed and validated an analytical method using an HPLC-PDA system with Inertsil C8 (150 x 4.6 mm, 5 µm) as the column. Therefore, this study chose HPLC-PDA.

Previous study (Janchawee et al., 2007) The characteristics of mitragynine were reported in various chromatographic conditions, particularly in the mobile phase selection. With >80% methanol in the mobile phase, mitragynine elutes quickly but interferes with other peaks in the sample. With methanol composition < 80%, mitragynine was well separated from the polar interferences, but broader peaks were observed. The use of a short column (150 mm) resulted in a run time of <10 min but poor resolution; as such, the analysis was carried out using a long column (250 mm) with a total run time of 30 min.

In this study, various HPLC column and mobile phase systems were assessed. Among the chromatographic columns evaluated, i.e., Sunfire C18, 4.6 mm x 250 mm, 5 µm (Waters, MA, USA), Symmetry C8, 4.6 mm x 150 mm, 5 µm (Waters, MA, USA) and Atlantis T3 C18, 4.6 mm x 250 mm, 5 µm (Waters, MA, USA). With Sunfire C18, mitragynine was eluted rapidly and not well separated from the solvent peak. Furthermore, symmetry C8 can separate mitragynine from other peaks despite poor resolution and broader peaks. Atlantis T3 C18 provided the best chromatography separation and shape of mitragynine. Several mobile phase compositions of acetonitrile with 0.05% formic acid (adjusted to pH 5 with NaOH) were also evaluated, i.e., ACN - 0.05% formic acid 50:50 (v/v), 65:35 (v/v), 70:30 (v/v), and 75:25 (v/v). The results indicated that the optimal mobile phase was ACN - 0.05% formic acid, 75:25 (v/v). It gave the best resolution and peak shape, while the total run time was 8 minutes. So, the column used in the study, T3 C18, differs from previous studies by Janchawee et al., 2007; Mudge & Brown, 2017; Parthasarathy et al., 2013. This aims to provide another alternative column that also has good performance.

3.2. Method validation

The method was selective and specific, in which the chromatographic peak of mitragynine was free of interferences originating from the sample matrix and blank. This was confirmed through the PDA chromatogram's peak purity curve, which evaluates the consistency of UV spectra at the start and end peaks. The purity curve shows that impurity was not detected with a peak purity index of 1.000000 for 7-hydroxy mitragynine (Fig. 3) and mitragynine (Fig. 4). Therefore, there are no interferences at retention time for both analytes. Furthermore, their peak profile was similar for 7-hydroxy mitragynine and mitragynine.

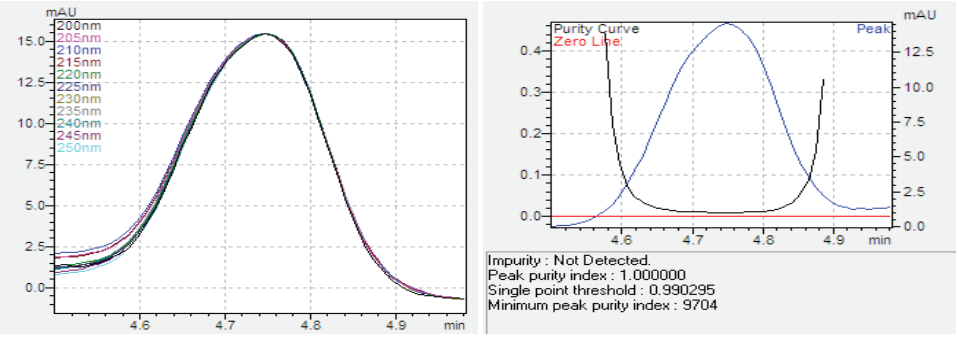


Figure 3. Purity curve and peak profile 7-hydroxy mitragynine at a variable wavelength

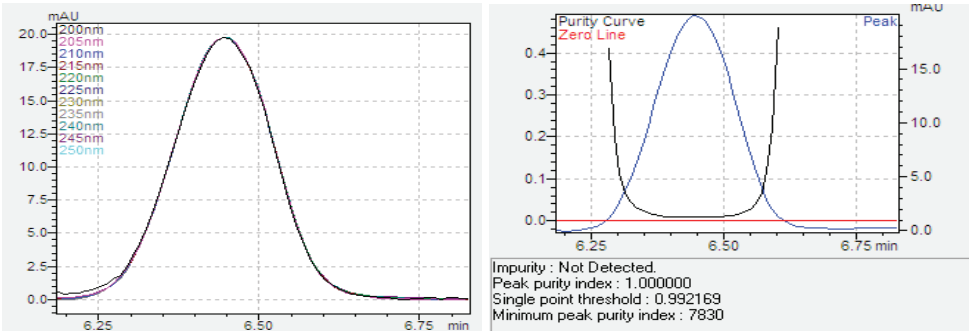
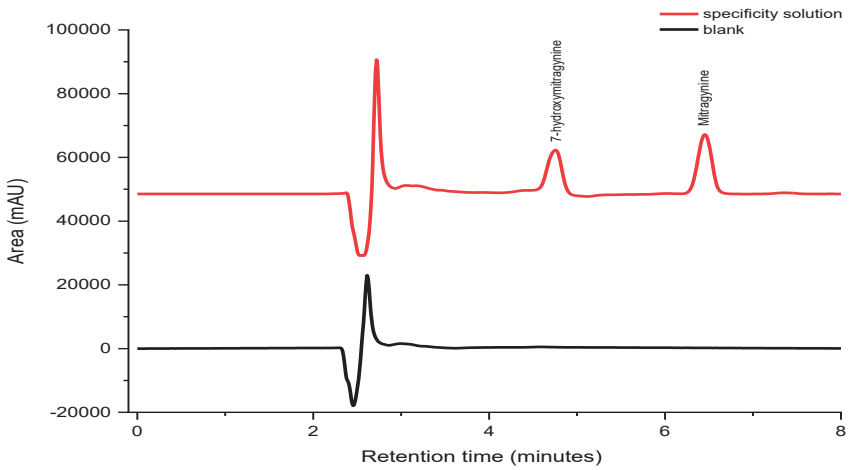


Figure 4. Purity curve and peak profile mitragynine at a variable wavelength

In Fig. 5, blank, 7-hydroxy mitragynine, and mitragynine peaks were well separated with a retention time of 2.5 minutes, 4.74 minutes, and 6.44 minutes, respectively. This method produced the best resolution between 7-hydroxy mitragynine and mitragynine at 5.58. In previous studies, hydroxy mitragynine was not used to see whether the separation conditions were good. This means that the method demonstrated its best ability to separate 7-hydroxy mitragynine, mitragynine, and other alkaloids in kratom samples. Moreover, the short analytical run time in this study is an advantage for routine detection and quantification of mitragynine in kratom products.



Peak	Retention time (minute)	Area	Tailing factor	Theoretical plate	Resolution
7-hydroxy mitragynine	4.744	151727	0.928	3903	--
Mitragynine	6.439	206295	1.011	7213	5.58

Figure 5. Blank, 7-hydroxy mitragynine, and mitragynine chromatogram

Identification was carried out by comparing the UV spectrum of the analyte with that of the mitragynine standard within 210 - 400 nm (Fig. 6). A representative chromatogram of the standard and samples is given in Fig. 11. The calibration curve of mitragynine was found to be linear over the concentration range of 1.959 - 6.010 µg/mL with a mean equation of y = 40,933.2072x + 3,747.2000 with R^2 of 0.9992 and Vx_0 value of 1.4%. The calculated lower limit of detection and quantitation were 0.14 µg/mL and 0.45 µg/mL, respectively. The sensitivity was higher than the previously reported methods. (Fowble & Musah, 2019; Parthasarathy et al., 2013) To analyze mitragynine in Kratom and its products. The range and limit of quantification were satisfactory for quantifying the level of mitragynine in the isolate and kratom extract. The precision and suitability system of the method was satisfied with the result in Table 1.

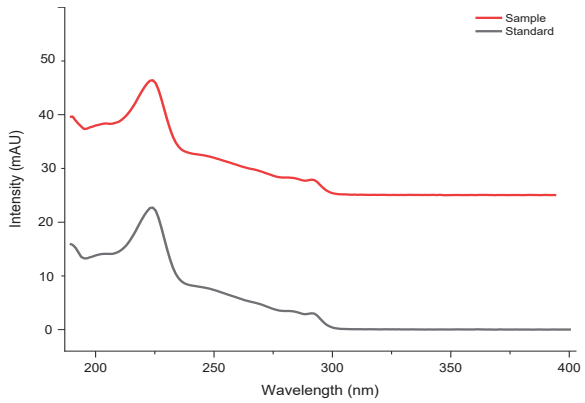


Figure 6. UV spectrum of standard and analyte in kratom sample which have a retention time that corresponds to mitragynine peak

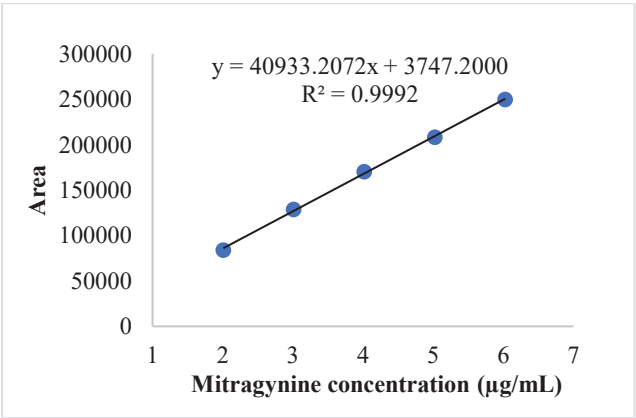


Figure 7. Linearity curve of standard mitragynine and sample (30:70)

Resolution between peaks due to 7-hydroxy mitragynine and mitragynine of 5.58 was well apart. The RSD of retention time and area at the system suitability solution was less than 2%. Repeatability for six determinations and intermediate precision were 0.69% and 1.57% at two consecutive days, respectively. The injection was replicated to ensure that the measured area or retention time was correct since the analysis results were valid. (USP, 2020). Suppose repeated injections produce variable area and retention time and do not meet the requirements; the resulting peak area of the chromatogram may be inappropriate, and the analysis will result in invalid data. The tailing factor and theoretical plate of the method also met the criteria.

Table 1. Precision and suitability system

Parameter	Criteria (USP, 2020)	Result	
		7-Hydroxymitragynine	Mitragynine
Resolution between 7-Hydroxymitragynine and Mitragynine	≥ 1.5	-	5.58
% RSD of retention time	≤ 2%	0.11	0.14
% RSD area	≤ 2%	0.31%	0.09%
Tailing factor	≤ 2	0.93	1.01
Theoretical plates	≥ 2000	3903	7213
Repeatability (n = 6)	≤ 2%	-	0.69% and 1.27%
Intermediate precision (2 days consecutive)	≤ 2%	-	1.54 %

This study's accuracy was measured by recovering the mitragynine standard in the spiked sample (30:70) and calculating the percent bias of the mitragynine standard using a calibration curve and certificate. The recovered mitragynine standard in the spiked sample ranged from 99.54% to 100.54%, and the percent bias (accuracy) was 0.27%. The percentage recovery and bias requirements were 98-102% and did not exceed 2%, respectively. (Ahuja, 2005; AOAC, 2002). It indicates that the methodology was accurate and valid for quantifying mitragynine in the kratom sample.

Table 2. Accuracy and recovery of mitragynine

Concentration (µg/mL)	% Recovery
3	99.64
4	100.54
5	99.79
Mean	99.49
% RSD	0.48
Bias (%)	0.27

In this study, robustness was in dire straits of analytical technique capability to stay unaffected. However, deliberate variations in technique parameters show its responsibility throughout normal usage. (ICH, 2022). Parameters were used, such as flow rate variation (0.8 mL/min, 1.0 mL/min, and 1.2 mL/min) and wavelength (223 nm, 225 nm, and 227 nm). The data were statistically analyzed using the Anova single factor. Robustness results are shown in Fig. 8 and Table 3 since Fcal < F crit was provided. This signifies that the change in flow rate and wavelength on mitragynine quantification using HPLC-PDA did not

significantly affect the assay of mitragynine. The analytical method for the quantification of mitragynine was robust.

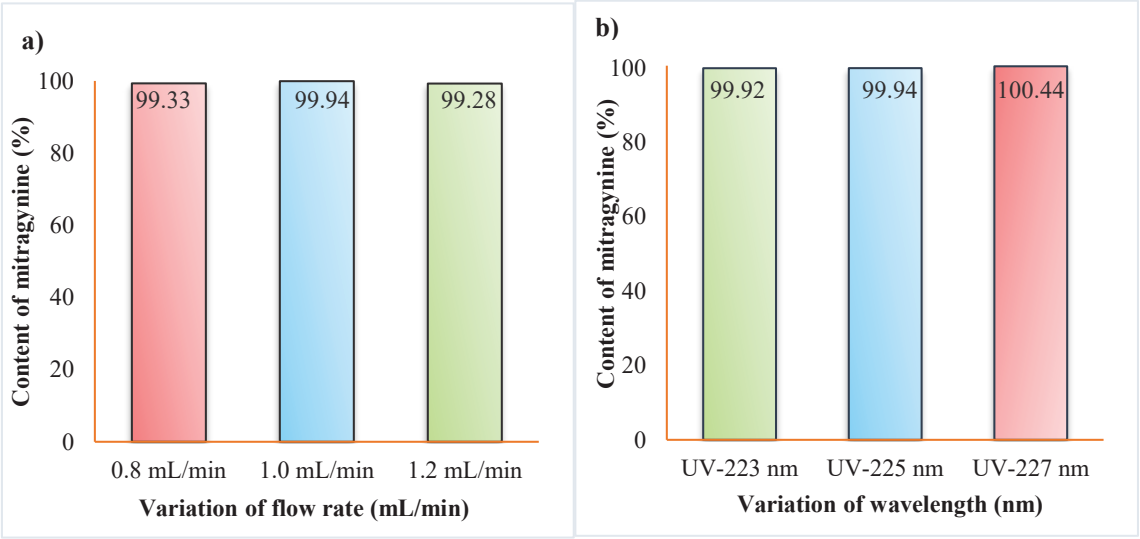


Figure 8. Robustness mitragynine analytical method with a). variable of flow rate; b). variable of wavelength

Table 3. *Anova single factor* robustness for quantification of mitragynine

Parameter	Fcalculated	Frit	P-value
Flow rate (±0.2 mL/min)	0.436149	5.143253	0.6655
Wavelength (±2 nm)	0.595003	5.143253	0.5811

3.3. Stability study of mitragynine

The result of the short-term stability study showed that mitragynine was stable in methanol at room temperature (24 - 26ºC) over 54 hours (Fig. 9). Statistical analysis with a single Anova factor demonstrated Fcal < Fcrit (2.260 < 2.604) for the 1 – 54-hour stability study. However, after 54 hours, the concentration of mitragynine decreased, resulting in stability tests of over 60 hours, Fcal > Fcrit (2.672 > 2.507).

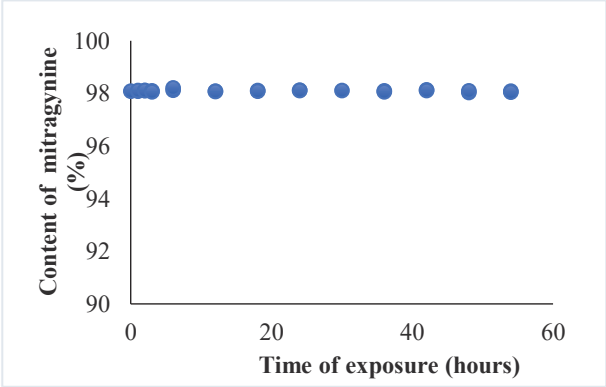


Figure 9. Short-term stability study of mitragynine in methanol solution

A long-term stability study of mitragynine in methanol solution at four ºC was reported by Parthasarathy et al., where mitragynine was stable over one month (Parthasarathy et al., 2013).

3.4. Sample analysis

This method was used to determine and quantify mitragynine in the kratom samples obtained from Putussibau, Kapuas Hulu, West Kalimantan Province, Indonesia, in 2020. Assay of mitragynine in Kratom using mitragynine standard and isolate. Fig. 10 shows a similar slope for both calibration curves. This means the calibration curve between the standard and the isolate was comparable. Analytical statistics using Anova single factor was obtained for Fcal < Fcrit (1.302 < 7.709) and a P-value of 0.318 > 0.05. Therefore, the mitragynine content in kratom material using standards and isolates was not significantly different.

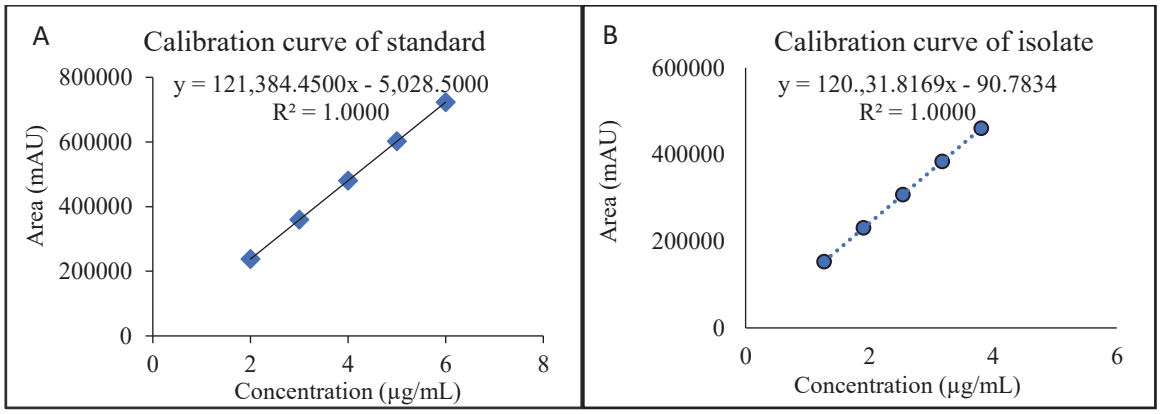


Figure 10. Calibration curve of standard (A) and isolate mitragynine (B)

The result (Table 4) showed that mitragynine content of 6.01 - 6.31 mg/g corresponded to the Prozialeck et al. (2020) study, where the content in kratom products and their derivatives varies in the range of 3.9 - 62.1 mg/g (Prozialeck et al., 2020) And commercial kratom product extracted by ultrasonication was 0.8 - 62.6 mg/g (Kikura-Hanajiri et al., 2009). The mitragynine content obtained in this study was lower than in previous studies because the samples used were in dried leaf form.

Table 4. Mitragynine content in kratom samples

Kratom sample	Mitragynine standard		Isolate	
	Mitragynine concentration (µg/mL)	Amount of mitragynine (mg/g)	Mitragynine concentration (µg/mL)	Amount of mitragynine (mg/g)
1	3.59	6.12	3.57	6.01
2	4.15	6.19	4.13	6.10
3	4.56	6.31	4.54	6.22

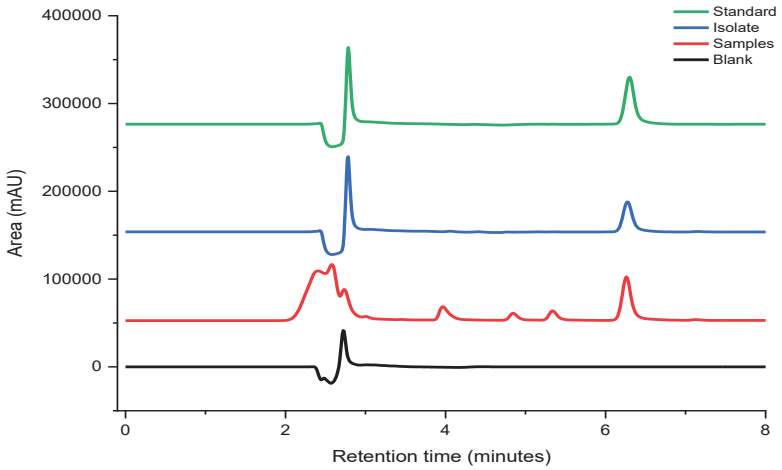


Figure 11. HPLC chromatogram, obtained on an Atlantis T3 C18 column (250 x 4,6 mm; 5µm) using a mobile phase of ACN – formic acid 0.05% pH 5.0 (75:25 v/v)

4. Conclusion

A simple, quick, and reliable HPLC-PDA method has been developed and validated to identify and quantify mitragynine in Mitragyna speciosa and its products. This method has an advantage over existing methods in terms of simplicity of sample preparation, short analysis time, and cost-effectiveness compared to GCMS and LCMS/MS. Therefore, it is suitable for routine screening of M. speciosa products in the market, especially Kratom materials. The amount of mitragynine in the Kratom samples obtained ranged from 6.01 to 6.31 mg/g.

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