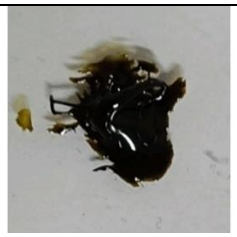
SUPPLEMENTARY FILES

Pengembangan Metode Non-Kromatografi untuk Pemurnian Mitraginin dari Daun Kratom (*Mitragyna speciosa*) melalui Variasi Pelarut pada Ekstraksi Asam Basa

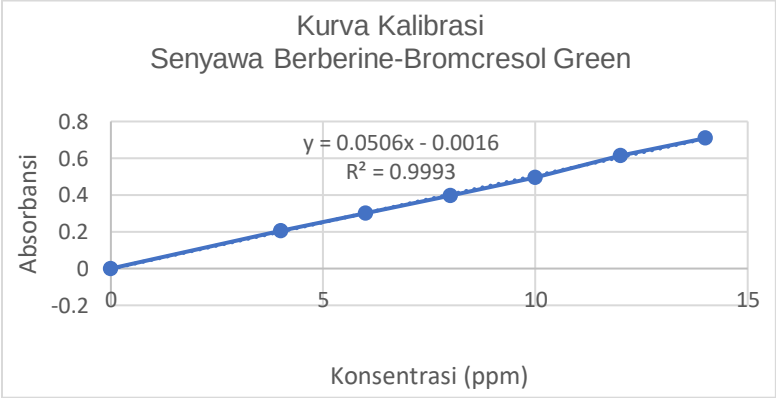
1. Hasil Maserasi Simplisia Daun Kratom Mitragyna speciosa (Metanol vs Etanol)

Jenis Ekstrak	Dokumentasi	Berat simplisia awal (g)	Berat ekstrak (g)	Rendemen (%)	Sifat Organoleptik
Ekstrak Metanol		100	24,4	24,4	Keduanya memiliki penampang fisik cokelat gelap kehijauan, berbau herba tajam, pahit, sedikit menyengat, dan kental
Ekstrak Etanol		100	13,9	13,9	

2. Perhitungan Total Alkaloid dengan *Bromcresol green*

Kurva Kalibrasi Bromcresol Green

Konsentrasi (ppm)	Absorbansi
0	0
4	0,206
6	0,3
8	0,397
10	0,495
12	0,615
14	0,709



Jenis Sampel	Absorbansi Sampel		X	Total Alkaloid (mg/g extract)	Mean	Standar Deviasi	Total Alkaloid ± SD
	Replikasi	Absorbansi					
Ekstrak Metanol	1	0,228	4,537549	9,075099	9,391304	0,447182	9,39 ± 0,447
	2	0,244	4,853755	9,70751			
Ekstrak Etanol	1	0,433	8,588933	17,17787	16,92095	0,363336	16,92 ± 0,363
	2	0,42	8,332016	16,66403			

Rumus

Persamaan garis kurva kalibrasi: y= 0.0506x - 0.0016

X = $\frac{y+0,0016}{0,0506}$ = C

Total Alkaloid Content = $\frac{C\ x\ Fp x\ V}{m}$

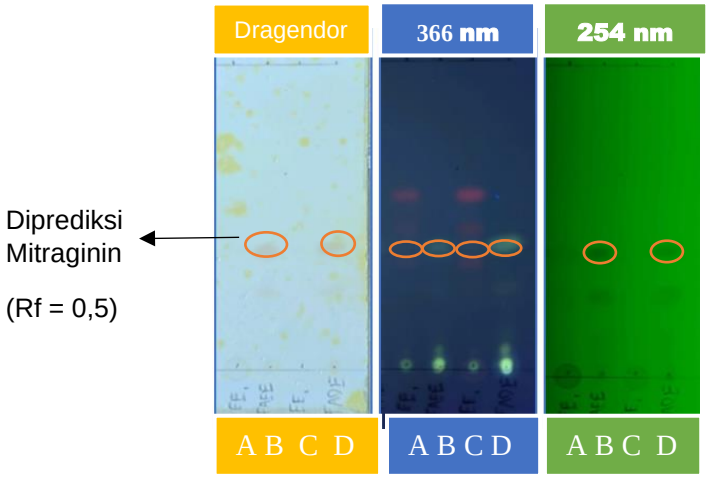
Keterangan:

Fp = 4

V = 0,01

Massa ekstrak = 0,02 gram

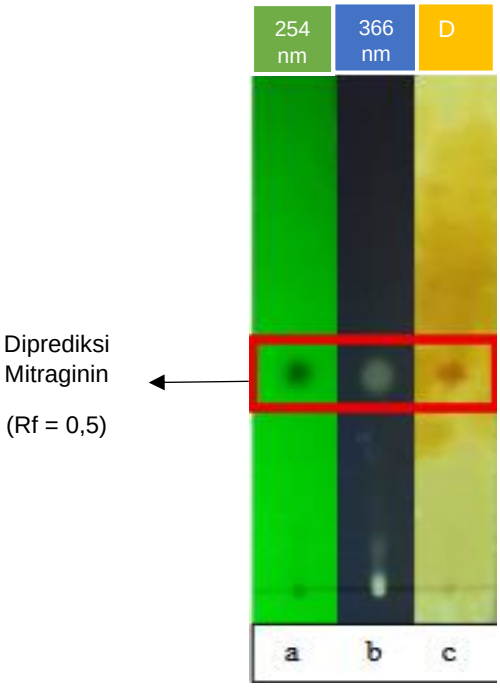
3. Profil Kromatografi Lapis Tipis *Mitragyna speciosa*
Metode ABBLE (Ekstraksi Asam-Basa Kombinasi Pelarut)



Keterangan:

- A : Ekstrak Etanol
- B : Fraksi Alkaloid EtOH-EtOAc
- C ; Ekstrak Etanol
- D : Fraksi Alkaloid EtOH-DCM

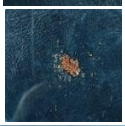
Metode Konvesional dengan Kromatografi Kolom Gravitasi



Keterangan:

- A : Profil noda pada panjang gelombang UV 254 nm
- B : Profil noda pada panjang gelombang UV 366 nm
- C :Profil noda setelah penyemprotan reagen dragendorf

4. **Hasil Ekstraksi ABBLE dan Kromatografi Konvensional**

	Kode Fraksi	Kadar mitraginin (%)	Rendemen (g)	Efisiensi proses (%)
	ABBLE EtOH-EtOAc	27,47	0,7022	19,29
	ABBLE EtOH-DCM	39,03	0,4855	19,06
	Kromatografi Konvensioal (Fraksi H)	47,9	0,2	9,58

Rumus Kadar Mitraginin (terhadap standar): = $\frac{\text{Konsentrasi teoritis}}{Konsentrasi\ sebenarnya}\ x\ (kadar\ standar\ mitraginin) x\ 100\%$

Rumus Efisiensi : $\frac{\text{massa mitraginin pada fraksi}}{massa\ mitraginin\ awal}\ x\ 100\%$

Massa mitraginin = Kadar mitraginin % x Rendemen (g)

5. Hasil Analisis Kadar Kemurnian Mitraginin dengan HPLC-UV dan Perhitungan Kadar Mitraginin

Kondisi Instrument:

Analisi dilakukan menggunakan instrumen *Agilent 1200 Series Liquid Chromatography* (Agilent, Mississauga, ON, Kanada) dengan detektor diode array, mengadaptasi metode Mudge & Brown [17]. Kolom analitik Kinetex 5 µm EVO C18 100A (150×4.6 mm, 5 µm partikel) digunakan pada suhu 25◦C. Detektor dipantau pada panjang gelombang 226 nm. Laju alir fase gerak diatur konstan pada 1.5 mL/menit dengan waktu alir 18 menit dan total siklus 22 menit. Volume injeksi sampel adalah 5 µL. Pemisahan dicapai menggunakan program elusi gradien dengan Fase Gerak A (5,0 mM buffer ammonium bikarbonat, pH 9,50) dan Fase Gerak B (Asetonitril). Kondisi gradien pelarut tertera pada Tabel. Standar referensi sekunder mitraginin (bersertifikat CRM) digunakan untuk membuat kurva kalibrasi (0,05-0,40 mg/mL) dengan memplot Luas Area (y) terhadap Konsentrasi (x). Persamaan garis dari kurva kalibrasi digunakan untuk menghitung kadar mitraginin.

Time, min	Pelarut A (%)	Pelarut B (%)	Laju alir (mL/min)
0	70	30	1,5
17	30	70	1,5
17,9	30	70	1,5
18	70	30	1,5

Perhitungan kadar mitraginin dalam sampel padat dilakukan dengan persamaan sebagai berikut.

$$\%\ (mitraginin) = (\frac{P_0 - b_0)}{m_0}\ \text{x}\ \frac{V}{W}\ x\ \frac{D}{1000}$$

Perhitungan kadar mitraginin dalm sampel cair dapat dikuantifikasi dengan persamaan berikut.

$$\%\ (mitraginin) = (\frac{P_0 - b_0)}{m_0}\ \text{x}\ D$$

Dimana P_0 = luas puncak kromatogram dari sampel target; b_0 = y-intersep dari kurva kalibrasi untuk analit, m_0 = kemiringan dari kurva kalibrasi mitraginin; V= volume dari larutan uji dalam mL; W = berat kering sampel (g); D = faktor pengenceran.

Hasil persentase kadar mitraginin dalam setiap sampel kemudian dikombinasikan dengan berat rendemen masing-masing fraksi untuk memperoleh persentase efisiensi proses. Berikut perhitungan persentase efisiensi proses untuk memperoleh mitraginin dan diastereoisomernya.

% efisiensi proses = % kadar mitraginin x berat rendemen fraksi alkaloid

BIOTEK REKAYASA INDONESIA

Office – Jalan Pemuda Nomor 33B, Jati, Pulogadung, Jakarta Timur 13220
Laboratory – CBD Niaga Sentul Blok D-1 Nomor 32, Citaringgul, Babakan Madang, Bogor 16810

Certificate of Analysis

Report Number	:	006-06/BIOTEK/VI/2025
COC ID	:	COC.250515-006
Number of Pages	:	6 Including Cover
Customer Sample(s) Identity	:	BRI-PR0525-0016 until BRI-PR0525-0017
Sampled by	:	~~Laboratory~~ / Customer
Priority	:	Normal / ~~Urgent~~
Date of Sample(s) Received	:	26th May 2025
Date of Analysis Finished	:	03rd June 2025
Customer	:	Saniya almasa
Customer Category	:	~~Company~~ / ~~Government Institution~~ / ~~School/University~~ Private Individual / ~~Others~~
Customer Contact Person	:	*Saniyaalmasa19@gmail.com*

Approved by,

BIOTEK REKAYASA INDONESIA

Firmansyah Karim
Director
Tuesday, 03rd June 2025

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• (1) is sign for subcontracted test parameter; • (2) is sign for non accredited test parameter or test parameter become unaccredited due to different sample matrix; • (3) is sign for parameter related to sample(s) taken not in accordance with the stated method as described in point d; (4) is sign for in situ test parameter; • NA mean Not Available or Not Applicable

Certificate of Analysis

I. Sample Description

1.1 Subject : Alkaloid Ekstrak Etanol

1.2 Batch Number : -

1.3 Expired Date : -

1.4 Lab Sample ID : BRI-PR0525-0016

1.5 Matrix : Liquid

II. Results

No.	Parameters	Units	Results	Method
1.	Mitragynine	%	27,47	HPLC

Certificate of Analysis

I. Sample Description

1.1 Subject : Alkaloid Ekstrak Metanol
1.2 Batch Number : -
1.3 Expired Date : -
1.4 Lab Sample ID : BRI-PR0525-0017
1.5 Matrix : Liquid

II. Results

No.	Parameters	Units	Results	Method
1.	Mitragynine	%	19,03	HPLC

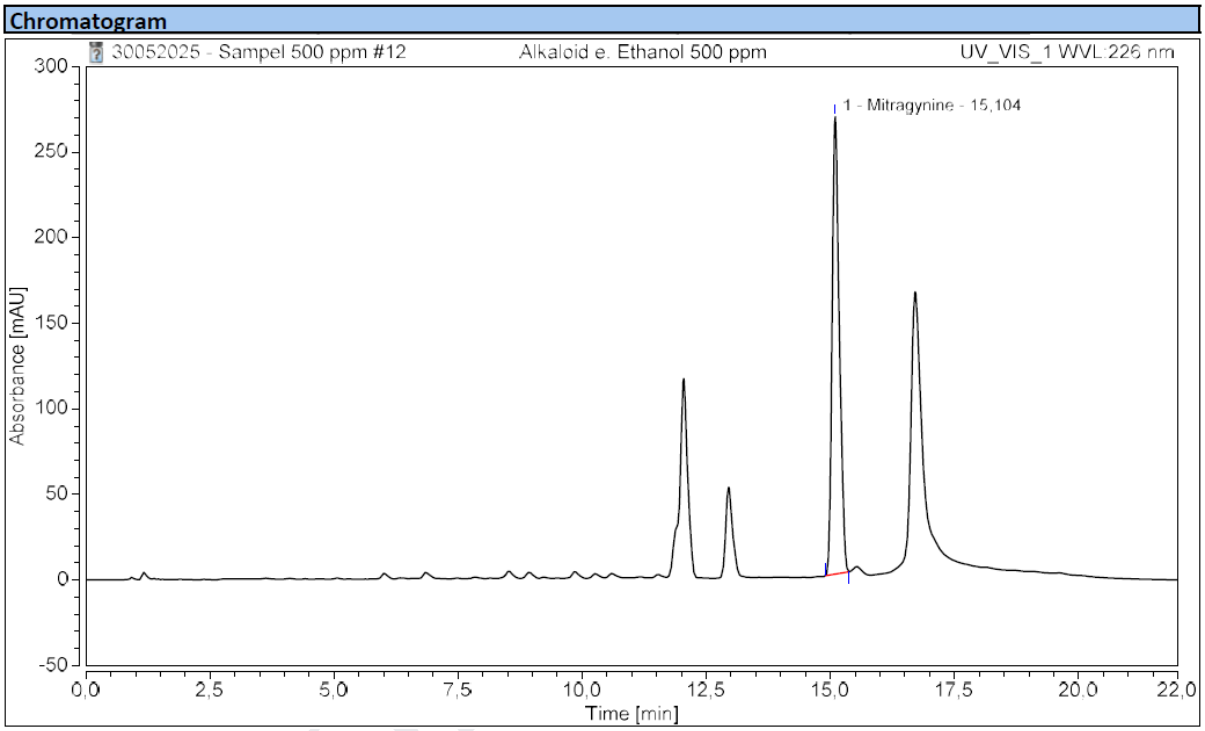
Approved by,

BIOTEK REKAYASA INDONESIA

Firmansyah Karim
Director
Tuesday, 03rd June 2025

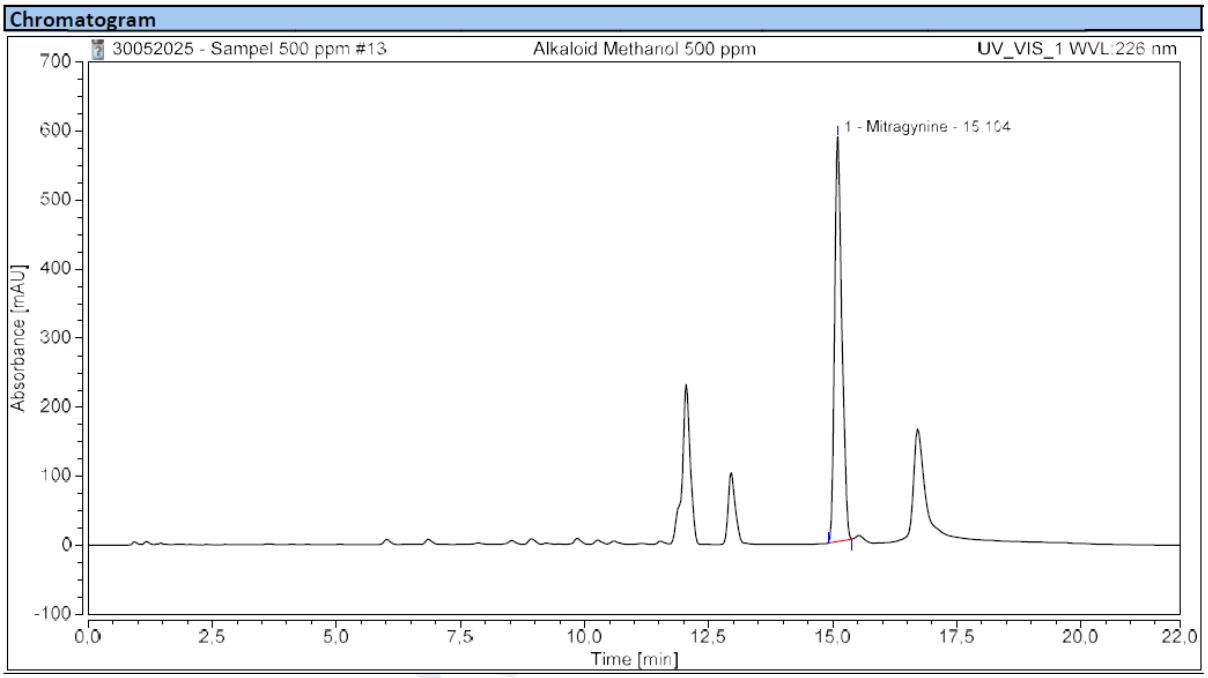
Lampiran 1.

Lab Sample ID : BRI-PR0525-0016



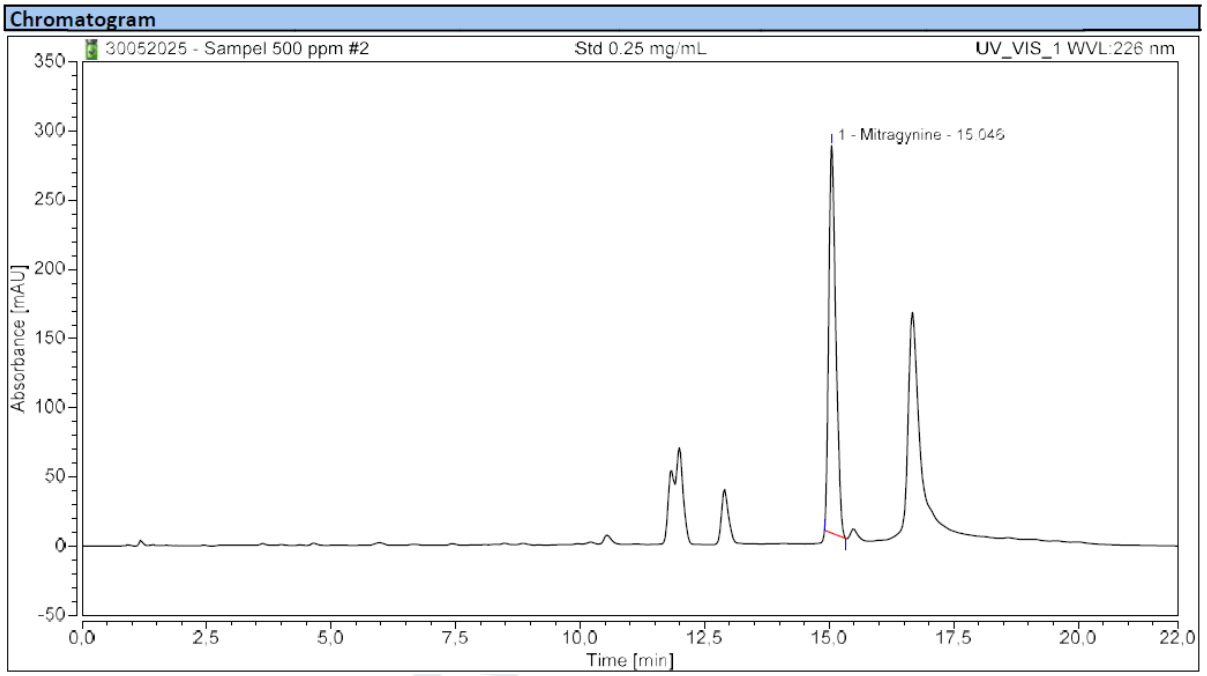
Lampiran 2.

Lab Sample ID : BRI-PR0525-0017

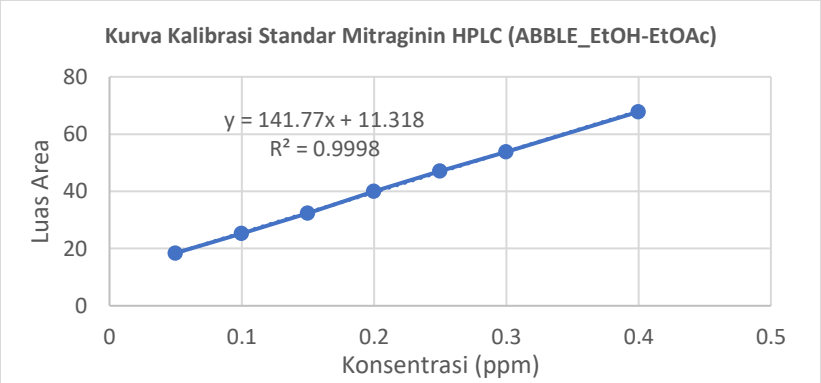


Lampiran 3.

Standar Mitragynine 0.25 ppm



a. Perhitungan Hasil Analisis Kadar Mitraginin Metode ABBLE (EtOH-EtOAc)



Konsentrasi (ppm)	Luas Area
0,05	18,437
0,1	25,253
0,15	32,364
0,2	40,062
0,25	47,004
0,3	53,864
0,4	67,803

Sampel	**Luas Daerah**	**Kadar Mitraginin**
Standar Senyawa Mitraginin Tersertifikat	49,290	57,6%
Fraksi ABBLE (EtOH-EtOAc)	45,060	27,4%

Y = 141,76x + 11,319

141,76 x = y - 11,319

141,76 x = 45,060 – 11,319

141,76 x = 33,741

X = 0,2380149548548532

Konsentrasi sebenernya = 500 mg/mL

Kadar mitraginin terhadap standar = $\frac{\text{Konsentrasi teoritis}}{Konsentrasi\ sebenarnya}\ x\ 100\%$

$= \frac{0{,}2380149548548532\ \text{ppm}}{0{,}5\ ppm}\ x\ 100\%$

= 47,60% dari standar sekunder

Kadar standar sekunder mitraginin yang digunakan= 57,6%

Kadar mitraginin dalam ekstrak = kadar mitraginin terhadap standar x kadar standar mitraginin x 100%

= 47,6 x 57,6 x 100%

= 27,4 %

Berdasarkan perhitungan di atas, diperoleh bahwa kadar mitraginin murni dalam Fraksi Alkaloid Etil Asetat Ekstrak Etanol (FAEE) sebesar 27,4 %.

BIOTEK REKAYASA INDONESIA

Office – Jalan Pemuda Nomor 33B, Jati, Pulogadung, Jakarta Timur 13220
Laboratory – CBD Niaga Sentul Blok D-1 Nomor 32, Citaringgul, Babakan Madang, Bogor 16810

Certificate of Analysis

Report Number	:	001/CERT-BRI/VI/2025
COC ID	:	COC.250605-001
Number of Pages	:	4 Including Cover
Customer Sample(s) Identity	:	BRI-IDS0525-0001
Sampled by	:	~~Laboratory~~ / Customer
Priority	:	Normal / ~~Urgent~~
Date of Sample(s) Received	:	05th June 2025
Date of Analysis Finished	:	13th June 2025
Customer	:	Saniya almasa
Customer Category	:	~~Company~~ / ~~Government Institution~~ / ~~School/University~~ Private Individual / ~~Others~~
Customer Contact Person	:	*Saniyaalmasa19@gmail.com*

Approved by,

BIOTEK REKAYASA INDONESIA

Firmansyah Karim
Director
Friday, 13th June 2025

Certificate of Analysis

I. Sample Description

1.1	Subject	: Alkaloid Ekstrak
1.2	Batch Number	: -
1.3	Expired Date	: -
1.4	Lab Sample ID	: BRI-IDS0525-0001
1.5	Matrix	: Powder

II. Results

No.	Parameters	Units	Results	Method
1.	Mitragynine	%	39,32	BRI-IK-LAB-006V01 (HPLC)

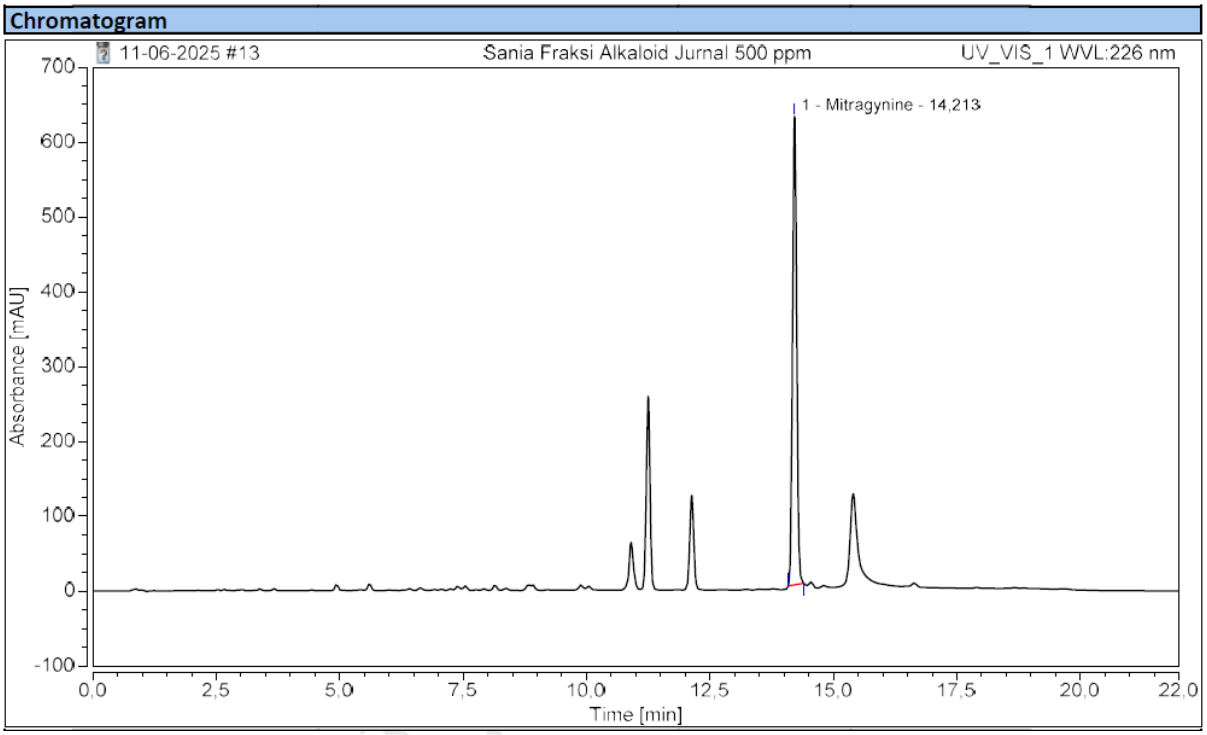
Approved by,

BIOTEK REKAYASA INDONESIA

Firmansyah Karim
Director
Friday, 13th June 2025

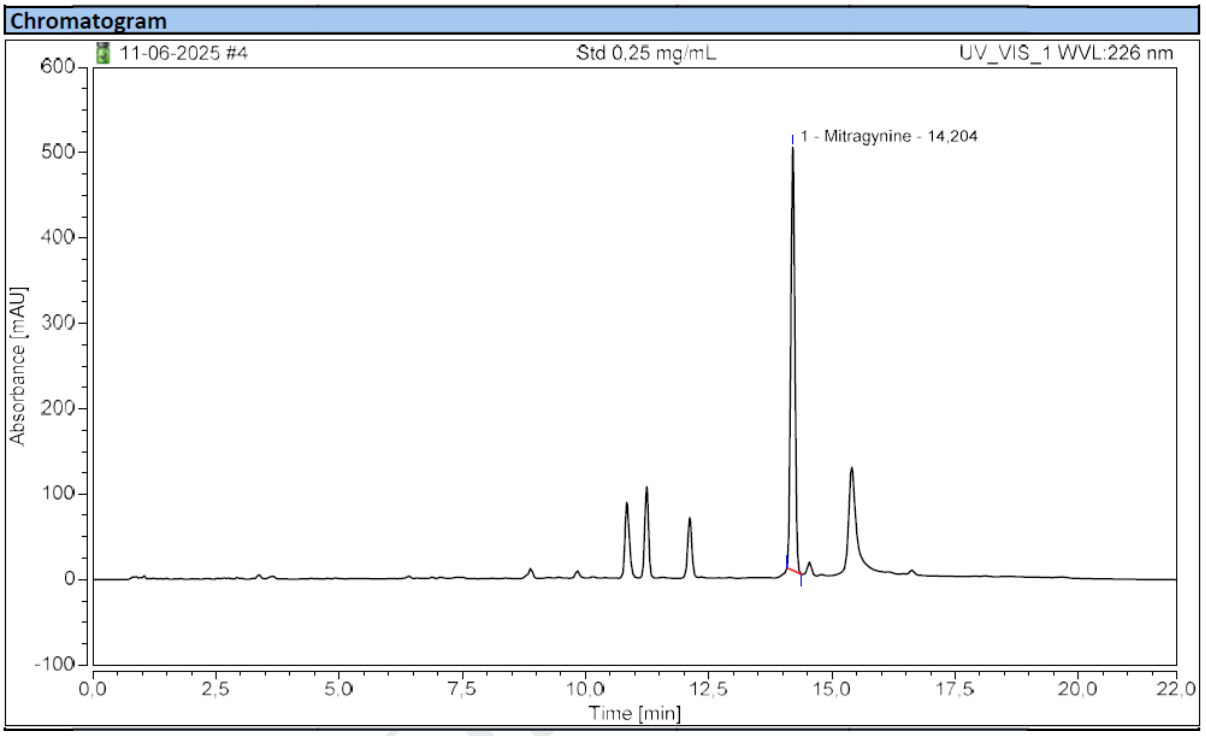
Lampiran 1.

Lab Sample ID : BRI-IDS0525-0001

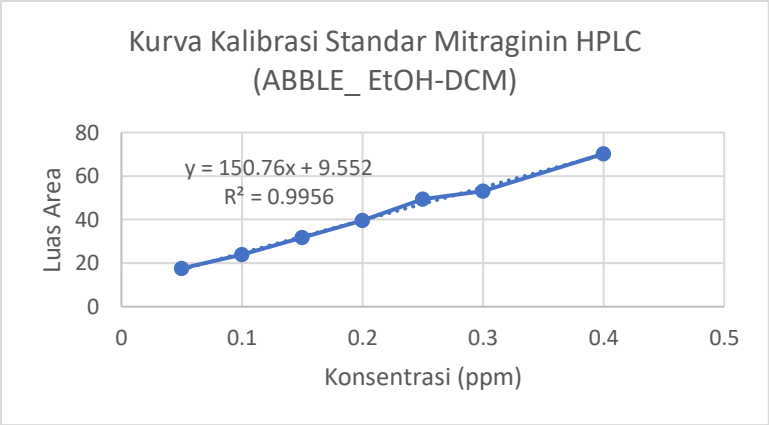


Lampiran 2.

Standar Mitragynine 0.25 mg/mL



b. Perhitungan Hasil Analisis Kadar Mitraginin Metode ABBLE (EtOH-DCM)



Konsentrasi (ppm)	Luas Area
0,05	17,5830
0,1	23,9870
0,15	31,8480
0,2	39,5270
0,25	49,3600
0,3	52,9440
0,4	70,2130

Sampel	Luas Daerah	Kadar Mitraginin (%)
Standar Senyawa Mitraginin Tersertifikat	49,290	57,6%
Fraksi ABBLE (EtOH-DCM)	60,926	39,03

y = 150,76x + 9,5516

150,76x = y – 9,5516

150,76x = 60,926 – 9,5516

150,76 x = 51,3744

X = 0,34076

Konsentrasi sebenernya = 500 ppm = 0,5 mg/mL

Kadar mitraginin terhadap standar $= \frac{\text{Konsentrasi teoritis}}{Konsentrasi\ sebenarnya}\ x\ 100\%$

$= \frac{0{,}34076\ \text{mg/mL}}{0{,}5\ mg/mL}\ x\ 100\%$

= 68,15 % dari standar sekunder

Kadar standar sekunder mitraginin yang digunakan= 57,6%

Kadar mitraginin dalam ekstrak = kadar mitraginin terhadap standar x kadar standar mitraginin x 100%

= 65,38% x 57,6% x 100%

= 39,03%

Berdasarkan perhitungan di atas, diperoleh bahwa kadar mitraginin murni sebesar 39,03 %.

BIOTEK REKAYASA INDONESIA

Office – Jalan Pemuda Nomor 33B, Jati, Pulogadung, Jakarta Timur 13220
Laboratory – CBD Niaga Sentul Blok D-1 Nomor 32, Citaringgul, Babakan Madang, Bogor 16810

Certificate of Analysis

Report Number	:	003/CERT-BRI/VI/2025
COC ID	:	COC.250605-003
Number of Pages	:	4 Including Cover
Customer Sample(s) Identity	:	BRI-IDS0525-0004
Sampled by	:	~~Laboratory~~ / Customer
Priority	:	Normal / ~~Urgent~~
Date of Sample(s) Received	:	12th June 2025
Date of Analysis Finished	:	13th June 2025
Customer	:	Saniya almasa
Customer Category	:	~~Company~~ / ~~Government Institution~~ / ~~School/University~~ Private Individual / ~~Others~~
Customer Contact Person	:	*Saniyaalmasa19@gmail.com*

Approved by,

BIOTEK REKAYASA INDONESIA

Firmansyah Karim
Director
Friday, 13th June 2025

Certificate of Analysis

I. Sample Description

1.1	Subject	: Mitragyna Speciosa Sampel Mitragyna .s.
1.2	Batch Number	: -
1.3	Expired Date	: -
1.4	Lab Sample ID	: BRI-IDS0525-0004
1.5	Matrix	: Powder

II. Results

No.	Parameters	Units	Results	Method
1.	Mitragynine	%	47.98	BRI-IK-LAB-006V01 (HPLC)

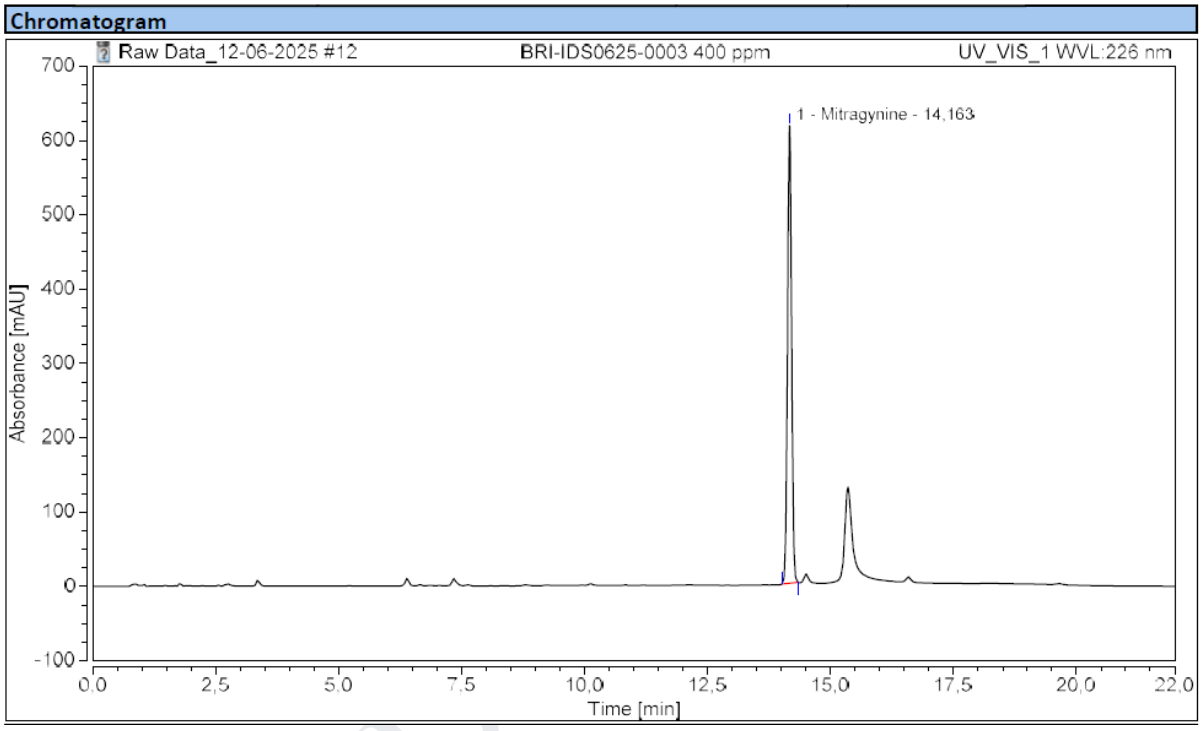
Approved by,

BIOTEK REKAYASA INDONESIA

Firmansyah Karim
Director
Friday, 13th June 2025

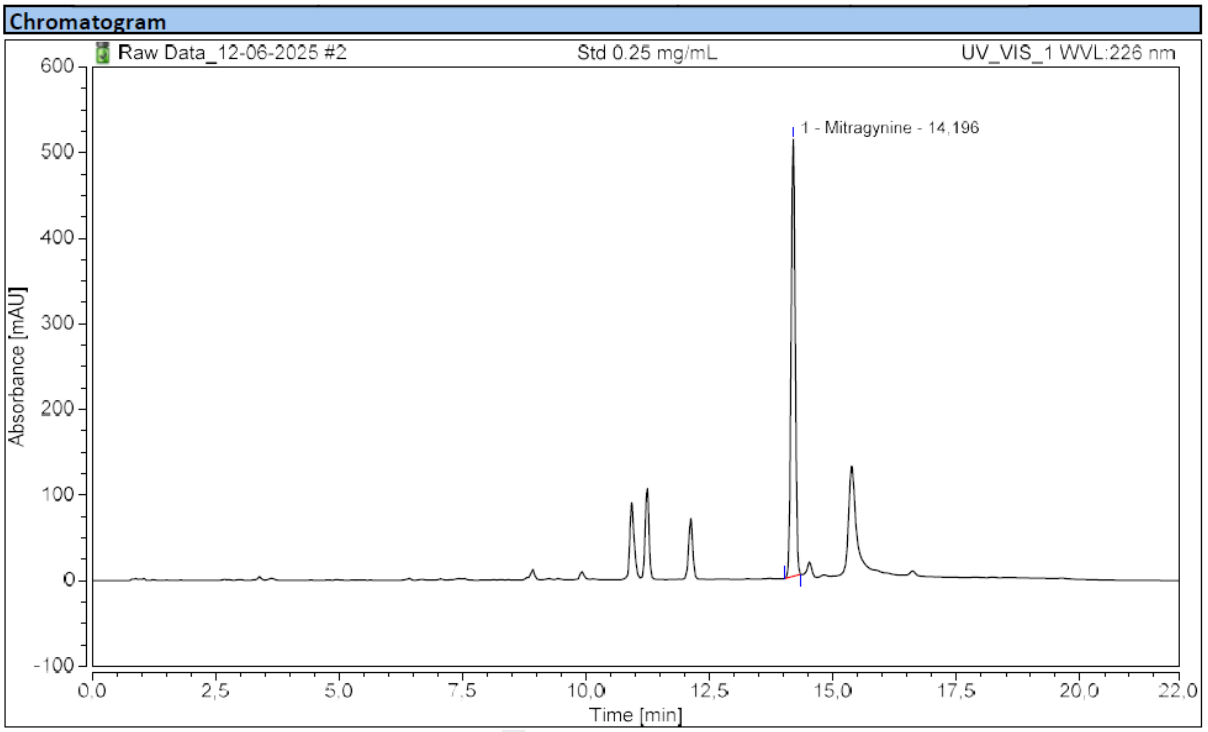
Lampiran 1.

Lab Sample ID : BRI-IDS0525-0001

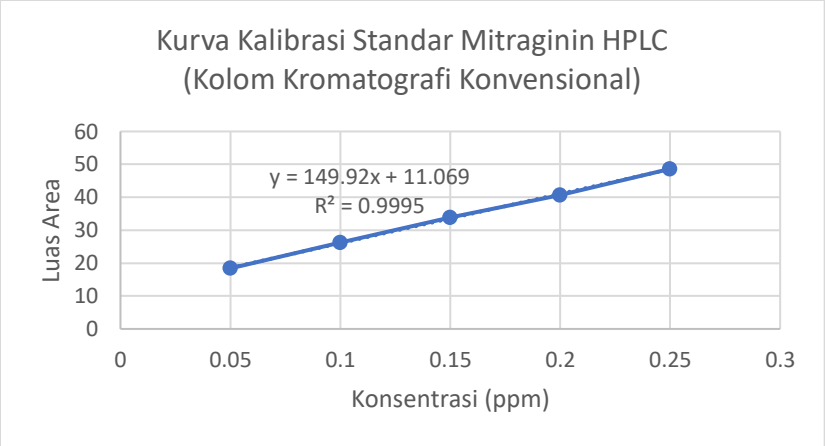


Lampiran 2.

Standar Mitragynine 0.25 mg/mL



c. Perhitungan Hasil Analisis Kadar Mitraginin Metode Kolom Kromatografi Konvensional



Konsentrasi (ppm)	Luas Area
0,05	18,402
0,1	26,202
0,15	33,848
0,2	40,698
0,25	48,633

Sampel	**Luas Daerah**	**Kadar Mitraginin (%)**
Standar Senyawa Mitraginin Tersertifikat	49,290	57,6%
Fraksi ABBLE (EtOH-DCM)	60,129	47,89%

y = 145,79 x + 11,641

145,79 x = y – 11,641

145,79x = 60,129– 11,641

145,79 x = 48,488

X = 0,3325

Konsentrasi sebenernya = 400 ppm = 0,4 mg/mL

Kadar mitraginin terhadap standar $= \frac{\text{Konsentrasi teoritis}}{Konsentrasi\ sebenarnya}\ x\ 100\%$

$= \frac{0{,}3325\ \text{mg/mL}}{0{,}4\ mg/mL}\ x\ 100\%$

= 83,125 % dari standar sekunder

Kadar standar sekunder mitraginin yang digunakan= 57,6%

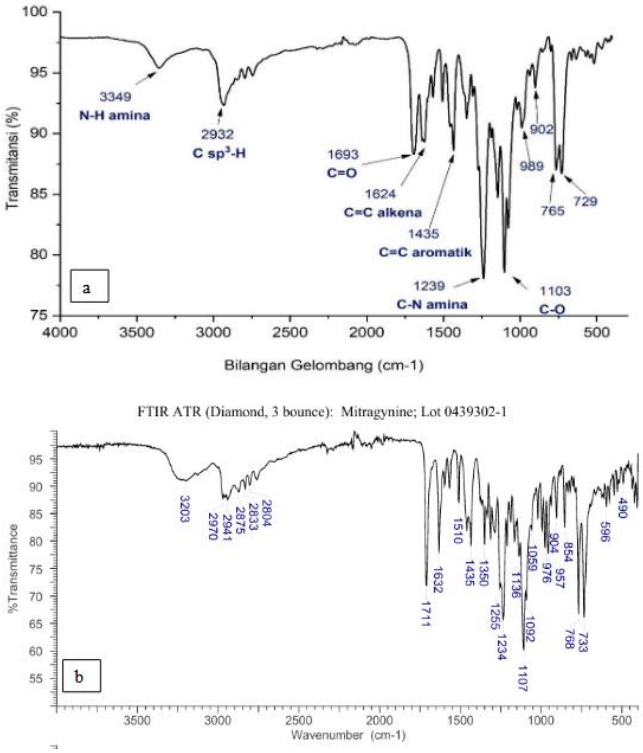
Kadar mitraginin dalam ekstrak = kadar mitraginin terhadap standar x kadar standar mitraginin x 100%

= 83,125 % x 57,6% x 100%

= 47,8%

Berdasarkan perhitungan di atas, diperoleh bahwa kadar mitraginin murni dalam Fraksi H Sebesar 47,8 %.

6. **Hasil Analisis FTIR Fraksi H Hasil Kolom Kromatografi Konvensional**



Gambar Spektrum FTIR Fraksi H (a.) Spektrum FTIR literatur (b)

Tabel Perbandingan bilangan gelombang fraksi H dengan literatur (senyawa standar mitraginin)

Gugus Fungsi	Spektrum Fraksi H (cm^{-1})	Spektrum Mitraginin Referensi (cm^{-1})	Keterangan
N-H (amina sekunder)	3349	3323	Serapan khas regangan N-H
C=O	1639	1711	Regangan karbonil ester lakton
C=C aromatik	1435	1435	Regangan cincin aromatik
C-N	1239	1234	Regangan C-N dari struktur amina
C-H aromatik	3015	±3000	Regangan C-H dari cincin aromatik

Spektrum FTIR antara fraksi H dan referensi literatur memiliki kesamaan yang menguatkan dugaan bahwa fraksi H mengandung mitraginin atau senyawa turunannya. Adanya serapan khas pada 3349 cm-1 dan 1239 cm-1 menegaskan keberadaan gugus amina sekunder, yang merupakan bagian penting dari struktur mitraginin, memiliki renggangan C-N pada serapan 1239 yang merupakan penanda gugus fungsi golongan alkaloid dan serapan kuat pada daerah 1639 cm-1 menunjukkan keberadaan gugus lakton yang bersifat khas pada mitraginin. Dengan demikian, spektrum FTIR fraksi H menunjukkan profil yang sangat mendekati mitraginin berdasarkan literatur.