Analysis of Mitragynine and 7-Hydroxymitragynine in Blood and Urine Using CLEAN-SCREEN XCEL® I and UHPLC-MS/MS

UCT Part Numbers

CSXCE103
Clean Screen® XCEL I
130 mg, 3mL Column

SPHACE5001-10
Select PH Buffer Pouches
100 mM Acetate pH 5.0

SLC-1850ID21-18UM
Selectra® C18 UHPLC Column
50 X 2.1 mm, 1.8 µm

SLC-18GDC20-18UMOPT
Selectra® C18 Guard Column
5 X 2.1 mm, 1.8 µm

SLGRDHLDR-HPOPT
Selectra® UHPLC
Direct-Connect Guard Holder

Summary:

Mitragynine is an indole-based alkaloid and the most abundant active alkaloid in the Southeast Asian evergreen tree Mitragyna Speciosa, commonly known as kratom. Kratom has been widely used in traditional medicine due to its opioid properties and stimulant-like effects[1]. Over 20 active alkaloids have been identified in kratom. Experts believe Mitragynine causes the opioid-like properties. Kratom has a strong bind to µ-opioid receptors, comparable to the scheduled opioid drugs [2]. 7-Hydroxymitragynine (7-OHMitragynine) is another active alkaloid present in kratom, although it is a minor constituent, and its opioid effects are weaker than Mitragynine effects.

There are currently no FDA-approved uses for kratom, and the agency has received concerning reports about the safety of kratom. Based on its action at the opioid receptors, kratom is often marketed as an alternative for pain relievers. In addition, some people take kratom to avoid the symptoms of opioid withdrawal and because kratom is easier to purchase than prescription drugs [2]. The FDA is actively evaluating all available scientific information on this issue and continues to warn consumers not to use any products labeled as containing the botanical substance kratom or its psychoactive compounds, Mitragynine and 7-Hydroxymitragynine [3].

This application note summarizes a quick, easy, and effective procedure to identify and quantitate Kratom analytes in blood and urine using Clean Screen® XCEL I SPE cartridges and a Selectra® C18 UHPLC column.

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SPE Procedure:

1. Sample Preparation

Urine Specimens:

To 1 mL of urine, add 1 mL of pH 5 Acetate buffer (0.1M) and internal standard(s).
Mix/vortex briefly.

Note: A hydrolysis protocol may be required if conjugated compounds are included in the above drug panel.

Blood Specimens:

To 1 mL of blood add 2 mL of pH 5 Acetate buffer (0.1M) and internal standard(s).
Mix/vortex briefly.
Centrifuge if necessary (e.g. postmortem blood).

2. Apply Sample

Load sample directly onto SPE column without conditioning (1-2 mL/minute).

3. Wash Cartridge

1 x 2 mL 1% Formic Acid in D.I Water
1 x 2 mL MeOH
Dry cartridges under full vacuum or pressure for 2 minutes.

4. Elute Analytes

1 x 2 mL MeOH: NH_4OH (98:2, v/v)
Collect at 1-2 mL/ minute.

5. Dry Eluate

Evaporate to dryness at < 40°C under a gentle stream of nitrogen.

6. Reconstitute

Reconstitute the sample in 500 µL of mobile phase or other appropriate organic solvents.

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LC-MS/MS Parameters

LC-MS/MS	Shimadzu LCMS-8050
UHPLC Column	Selectra® C18 (50 X 2.1 mm, 1.8 µm)
Guard Column	Selectra® C18 (5 X 2.1 mm, 1.8 µm)
Column Temperature	40°C
Flow Rate	0.4 mL/min
Injection Volume	5 µL

Gradient:

Time (min)	Mobile Phase A (%) (0.01% Formic Acid in Water)	Mobile Phase B (%) (Acetonitrile)
0	95	5
1.0	0	100
2.5	0	100
2.6	95	5
4.5	95	5

MRM Table:

Analyte	Parent Ion	Product Ion 1	CE	Product Ion 2	CE	Product Ion 3	CE
Mitragynine	398.90	174.05	33	238.10	26	226.05	25
Mitragynine-D3	401.90	177.05	33	238.05	26	226.10	26
7-OH-Mitragynine	414.90	190.00	30	175.05	48	397.10	26
7-OH-Mitragynine-D3	418.55	193.10	30	175.05	47	400.10	25

Chromatogram:

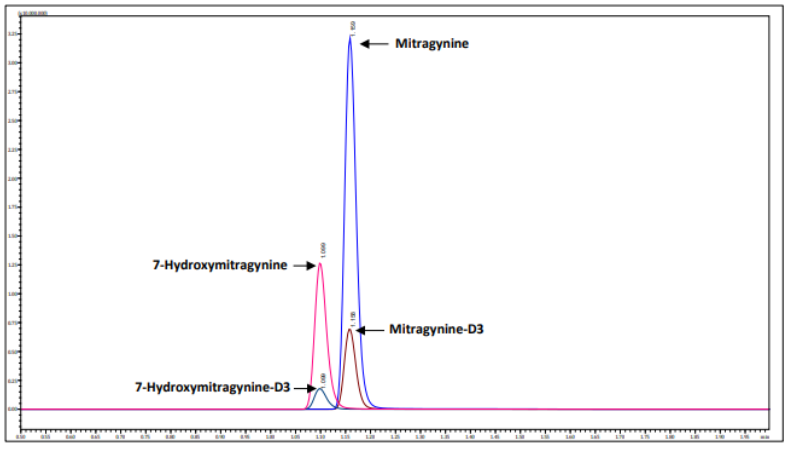

Figure 1: Chromatogram of a 25 ng/mL extracted urine sample.

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Calibration Curves:

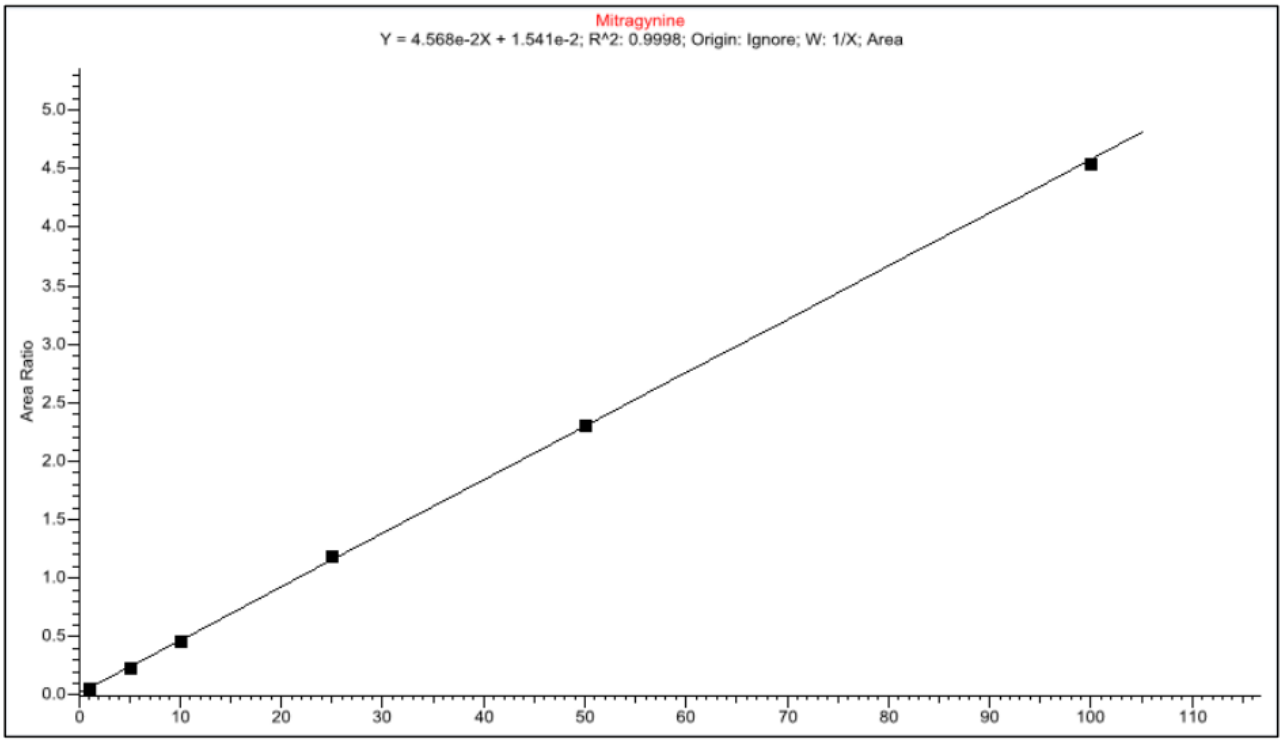

Figure 2: Calibration curve of Mitragynine (1, 5, 10, 25, 50, 100 ng/mL) featuring a R^2 of 0.9998.

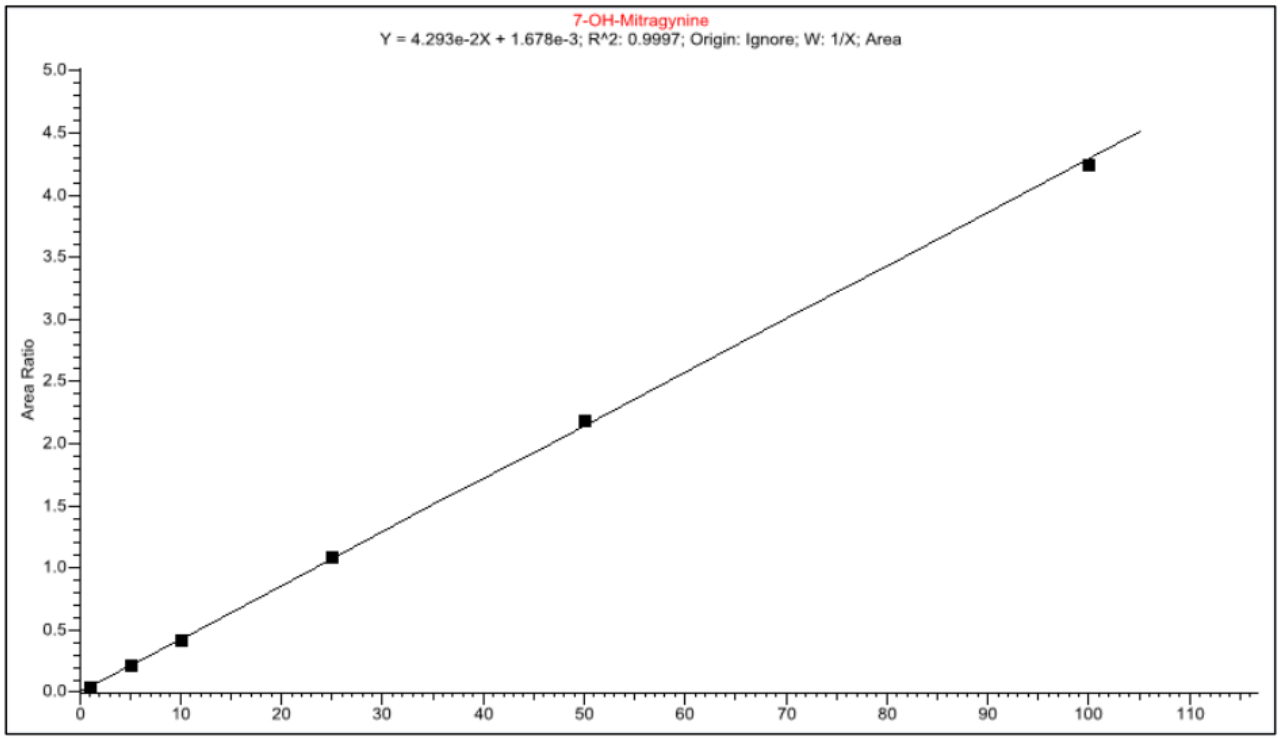

Figure 3: Calibration curve of 7-Hydroxymitragynine (1, 5, 10, 25, 50, 100 ng/mL) featuring a R^2 of 0.9997.

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Results:

URINE				
Analyte	**Mean Recovery (%) 2.5 ng/mL**	**RSD (%) (n=5)**	**Mean Recovery (%) 25 ng/mL**	**RSD (%) (n=5)**
Mitragynine	91	0.08	99	0.62
7-Hydroxymitragynine	96	0.03	97	0.55

BLOOD				
Analyte	**Mean Recovery (%) 2.5 ng/mL**	**RSD (%) (n=5)**	**Mean Recovery (%) 25 ng/mL**	**RSD (%) (n=5)**
Mitragynine	92	0.18	99	1.25
7-Hydroxymitragynine	100	0.12	99	1.30

Conclusions:

This application note outlines a simple SPE procedure for analyzing the active ingredients in Kratom, Mitragynine and 7-Hydroxymitragynine, in blood and urine, using UCT's Clean Screen® XCEL I SPE cartridges and Selectra® C18 UHPLC column. Excellent recoveries were obtained for both compounds using the outlined procedure, namely ≥90% at the 2.5 ng/mL level and ≥95% at the 25 ng/mL level. RSD values at both concentration levels were ≤2%. Chromatographic separation of the closely related analytes can be challenging due to the similar physicochemical properties of the two drugs. However, using a Selectra® C18 UHPLC column resulted in excellent retention and baseline separation of the two analytes in less than 1.5 minutes. Reproducible injections were obtained, and the peak width of all analytes in the methods did not exceed 0.1 min. Several forensic and clinical laboratories are targeting these analytes at a cut-off LOQ concentration of 10ng/mL. In this application note, we dropped the cut-off LOQ concentration to 1 ng/mL, which would be extremely useful for forensic and clinical diagnostics. This simple method will be beneficial to any lab looking to implement testing of these controversial drugs.

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References:

[1] "Kratom" National Center for Complementary and Integrative Health. Published November 2018. <https://www.nccih.nih.gov/health/kratom>.

[2] "Statement from FDA Commissioner Scott Gottlieb, M.D., on the agency's scientific evidence on the presence of opioid compounds in kratom, underscoring its potential for abuse." Food & Drug Administration. Published February 6, 2018. <https://www.fda.gov/news-events/press-announcements/statement-fdacommissioner-scott-gottlieb-md-agencys-scientific-evidence-presence-opioid-compounds>.

[3] "FDA and Kratom" Food & Drug Administration. Published September 11, 2019. <https://www.fda.gov/news-events/public-health-focus/fda-and-kratom>.

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