

PRESENTED AT THE 2018 SOCIETY OF FORENSIC TOXICOLOGISTS (SOFT) CONFERENCE,
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Development of a New Homogeneous Enzyme Immunoassay for the Detection of Mitragynine in Human Urine

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Background

Kratom, the common name for the plant *Mitragyna speciosa*, is commonly used in Southeast Asia for its opioid agonistic properties. The main alkaloids in Kratom include mitragynine, speciogynine, speciociliatine, paynantheine, and 7-hydroxymitragynine. The common route of ingestion consists of chewing or smoking Kratom leaves as well as drinking tea brewed using Kratom leaves. In the United States, Kratom can be purchased in various forms, including capsules, powders, e-liquid, and chocolate bars. Kratom is not a controlled substance, but is listed as a “drug of concern” by the U.S. Drug Enforcement Administration.

Objective

The objective of this study was to develop a new homogeneous enzyme immunoassay that can detect Mitragynine in urine using the CEDIA™ Technology. Further, the antibody will have minimal cross-reactivity to opiate compounds.

Materials and Methods

CEDIA Technology is based on the bacterial enzyme β -Galactosidase which has been genetically engineered into two inactive fragments, Enzyme Acceptor (EA) and Enzyme Donor (ED). These fragments spontaneously re-associate to form an active enzyme. In the absence of analyte from the sample, the specific antibody binds the ED-drug conjugate causing a decrease in enzyme activity. The free drug in the sample will compete for the limited number of antibody binding sites, making the ED-drug conjugate available for complementation to form an active enzyme. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is then determined spectrophotometrically at 570 nm.

The performance of the assay was evaluated on the Beckman Coulter AU680 analyzer. The Mitragynine assay uses a 50 ng/mL cutoff calibrator with controls at 50% of the cutoff. Patient samples were obtained from pain management laboratories. Method comparison and cross-reactivity studies were performed to determine the overall performance of the assay.

Results

Results presented below are from proof-of-concept studies

Table 1. Precision

Precision was carried out using Low and High Controls (25 & 75 ng/mL) and Cut-off Calibrator (50 ng/mL) in replicates of six. The samples were run in a random order, twice-a-day, over 5 days for a total of 60 replicates.

Qualitative Mode

Level	Mean (mA/min)	Within Run Precision		Total Run Precision	
		SD	%CV	SD	%CV
25 ng/mL	514	2.92	0.57	3.24	0.63
50 ng/mL	581	3.34	0.57	3.75	0.65
75 ng/mL	625	3.17	0.51	4.24	0.68

Semi-Quantitative Mode

Level	Mean (ng/mL)	Within Run Precision		Total Run Precision	
		SD	%CV	SD	%CV
25 ng/mL	26.7	1.29	4.85	1.30	4.87
50 ng/mL	52.9	2.30	4.34	2.52	4.77
75 ng/mL	80.2	2.93	3.66	3.01	3.76

Table 2. Cross-Reactivity

The cross-reactivity of the assay against Kratom alkaloids were tested.

Compound	Tested Concentration (ng/mL)	Result	Cross-Reactivity (%)
Paynantheine	25,000	Positive	0.2
7-hydroxy mitragynine	10,000	Negative	< 0.5

Table 3. Opiate and Opioid Cross-reactivity

Compound	Tested Concentration (ng/mL)	Result
6-Acetyl Morphine	100,000	Negative
Buprenorphine	100,000	Negative
Codeine	100,000	Negative
Dextromethorphan	100,000	Negative
Dihydrocodeine	100,000	Negative
EDDP	50,000	Negative
EMDP	100,000	Negative

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Compound	Tested Concentration (ng/mL)	Result
Fentanyl	100,000	Negative
Heroin	100,000	Negative
Hydrocodone	100,000	Negative
Hydromorphone	100,000	Negative
LAAM	100,000	Negative
Levorphanol	100,000	Negative
Meperidine	100,000	Negative
Methadone	100,000	Negative
Morphine	100,000	Negative
Morphine-3 β -D-glucuronide	100,000	Negative
Morphine-6 β -D-glucuronide	100,000	Negative
Nalbuphine	100,000	Negative
Nalorphine	100,000	Negative
Naloxone	100,000	Negative
Naltrexone	100,000	Negative
Norcodeine	100,000	Negative
Norhydrocodone	100,000	Negative
Noroxycodone	100,000	Negative
Noroxymorphone	100,000	Negative
Norpropoxyphene	100,000	Negative
Oxycodone	100,000	Negative
Oxymorphone	100,000	Negative
Propoxyphene	100,000	Negative
Tapentadol	100,000	Negative
Tramadol	100,000	Negative

Table 4. Non-Critical Cross-reactivity

Over-the-counter and commonly prescribed drugs, as well as illegal drugs, such as synthetic cannabinoids and bath salts, were tested for cross-reactivity in the Mitragynine assay.

Compound	Tested Concentration (ng/mL)	Result
11-nor- Δ^9 -THC-COOH	100,000	Negative
Acetaminophen	1,000,000	Negative
Acetylsalicylic acid	1,000,000	Negative
Amitriptyline	100,000	Negative
Amoxicillin	100,000	Negative
Amphetamine	100,000	Negative
Benzoyllecgonine	100,000	Negative
Caffeine	100,000	Negative
Chlorpromazine	100,000	Negative
Chloroquine	100,000	Negative
Cimetidine	100,000	Negative
Clomipramine	100,000	Negative
Desipramine	100,000	Negative
Digoxin	100,000	Negative
Diphenhydramine	500,000	Negative
Doxepine HCl	100,000	Negative
Ibuprofen	100,000	Negative
Imipramine	100,000	Negative
Methamphetamine	100,000	Negative
Norsertaline	100,000	Negative
Oxaprozin	50,000	Negative
Phencyclidine	100,000	Negative
Phenobarbital	100,000	Negative
Ranitidine	100,000	Negative
Secobarbital	100,000	Negative
Zaleplon	100,000	Negative
Zolpidem	100,000	Negative
Zopiclone	100,000	Negative

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Compound	Tested Concentration (ng/mL)	Result
AKB-48	100,000	Negative
5F-AB-PINACA	100,000	Negative
ADB-FUBINACA	100,000	Negative
AB-CHIMINACA	100,000	Negative
AB-FUBINACA	100,000	Negative
JWH-018	100,000	Negative
MDMB-CHMICA	100,000	Negative
UR-144	100,000	Negative
α -PVP	100,000	Negative
Buphedrone	100,000	Negative
Cathinone	100,000	Negative
Ethylone	100,000	Negative
Mephedrone	100,000	Negative
Methedrone	100,000	Negative
Methylone	100,000	Negative
Pentedrone	100,000	Negative

Table 5. Method Comparison

868 samples were tested by both Immunoassay and LC-MS/MS.
There was 1 borderline positive sample by immunoassay.

		LC-MS/MS	
		+	-
Mitragynine EIA	+	16	1 *
	-	0	851

* This sample was borderline positive in immunoassay.

Conclusions

The data presented here on the CEDIA Mitragynine (Kratom) Assay* demonstrates:

- Good precision
- Good specificity and sensitivity to Mitragynine
- Good correlation to LC-MS/MS
- No significant cross-reactivity to opiates, opioids, common over-the-counter drugs, prescribed drugs and other illegal drugs

Please refer to CEDIA Mitragynine (Kratom) Assay* Package Insert for updated performance results.

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Find out more at thermofisher.com/diagnostics

Assay is available April 2019. For *Criminal Justice and Forensic Use Only.

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