KRA-04

Method Title: LC/MS Method for the Identification of Mitragyna speciose (Kratom) and Quantitation of Mitragynine Using Linear Ion Trap Mass Spectrometer

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LC/MS Method for the Identification of Mitragyna speciose (Kratom) and Quantitation of Mitragynine Using Linear Ion Trap Mass Spectrometer

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INTRODUCTION

The FDA issued Import Alert 54-15 in response to the increasing occurrences of the importation of dietary supplements or bulk dietary ingredients that are or contain *Mitragyna speciosa* or Kratom. Products containing Kratom are adulterated because they contain an unapproved new dietary ingredient that has not been proven to be safe. Kratom can occur in a variety of forms including capsules, whole leaves, processed leaves, leaf resins, leaf extracts in powder or liquid form and powdered leaves(1). Of the many different alkaloids that are found in the leaves of the Kratom tree, mitragynine and 7-hydroxymitragynine are two of the most important ones. Several methods exist in which mitragynine is used as a marker compound for the identification of mitragynine (2-3). An LC/MS method is used to confirm the identity of suspect material as Kratom based on the comparative chromatographic profile and to quantify mitragynine and 7-hydroxymitragynine. The method has been applied to a variety of matrices: liquid drinks, liquid tinctures, powders, bulk ground processed leaves, dried leaves and capsules.
LC/MS Method – Memo of Analysis

EXPERIMENTAL

Suspect Kratom samples (0.1 to 0.5 g) are prepared using sonication for 30 minutes in 10 mL 50:50 acetonitrile/water. The extract is filtered and diluted for LC/MS analysis. The chromatography (Agilent 1200) uses a water + 0.1% formic acid / acetonitrile+ 0.1% formic acid gradient and a Zorbax SB-C18, 2.1 x 150 mm, 5µ column (Agilent). LC/MS experiments performed on a LTQ XL linear ion trap mass spectrometer (ThermoFisherScientific) are used to confirm the identity of the material using mitragynine and 7-hydroxymitragynine as chemical markers. Mitragynine is quantitated using the UV chromatogram and 7-hydroxymitragynine is quantitated using extracted ion chromatograms due to the much lower sample concentrations. In addition, the chromatographic profiles for several of the peaks in the sample are compared to the chromatographic profile of a reference material.

Memo of Analysis:
LC/MS instrument: PSW-GEN-E-0043, ThermoElectron LTQ XL, S/N: LTQ20573, FDA#5122576; Xcalibur, v 2.0 software, Agilent 1200 series HPLC with PDA detector, FDA#5122567. Calibration due 5/16.
Column: Zorbax SB-C18, 2.1 x 150 mm, 5μ, S/N USCN006051, P/N: 883700-922
Mobile Phase
A – Milli-Q water + 0.1% formic acid; B – Acetonitrile+ 0.1% formic acid

Gradient Program

Time	%A	%B
0	95	5
15	5	95
23	5	95
24	95	5

Flow rate 0.2 mL/min
1 μL injection volume
Column Heater at 40°C
post time 7 minutes
run time 30 minutes
UV detection at wavelength of 224

Mass Spectrometer

Ionization ESI	Sheath Gas Flow Rate 25	Aux/Sweep Gas Flow Rate 0
Source Voltage 5 kV	Capillary Temp 275°C	Tuned at m/z 524
Scanning m/z 110-1050 and Dependent Scan on most intense ion, collision energy 35		

CHROMADEX Standards:
Kratom Leaf Biological Reference Material - Kratom Balinese (Mitragyna speciosa) Leaf BRM, Lot 00031085-302
Mitragynine, 92.6%, $C_{23}H_{30}N_2O_4$, 398.50 [4098-40-2], Lot 00013890-9019

CERILLIANT Standards:
Mitragynine, , M-152, Lot FN-93-1401, 100 mg/mL in 1 mL methanol
7-Hydroxymitragynine, H-099, Lot FN10241402, 100 mg/mL in 1 mL methanol +0.1N ammonia

The major characteristic component of Kratom or *Mitragyna speciosa* is Mitragynine. Mitragynine is used to confirm the identity of a suspect material as Kratom.

Sample preparation

A (portion of the liquid)(portion of the powder)(portion of ground leaf)(contents of 10 capsules)(portion of tablet composite) was placed into a 20 mL scintillation vial and weighed. 10 mL of 50:50 acetonitrile/water was added to the vial. The sample was sonicated for 1minute and then filtered with 0.2 μm PTFE syringe filter. The filtered extracts were diluted for LC/ MS analysis.

The sample preparation was stored in the refrigerator when not being analyzed.

Method Blank – 10 mL acetonitrile/water taken through the sample preparation.

RESULTS

The chromatographic profile for unknown samples is compared to that for authentic *Mitragyna speciosa*. **Figure 1** and **Table 1** show the peaks that are observed in Kratom samples that are present in authentic material. Tentative identification has been assigned to most peaks based on literature reports (4-6). This chromatographic profile, as well as the presence of the marker compounds, mitragynine and 7-hydroxymitragynine, are used to confirm the identity of unknown samples. The experimentally determined amount of mitragynine in regulatory samples is shown in **Table 2**. 7-hydroxymitragynine was not quantitated in these regulatory samples. With each experimental batch of samples, a Kratom Biological Reference material extract was included as a positive control. The acetonitrile/water extract contains peaks not reported in the references which use a methanolic extraction.

Table 1. Tentative ID and mass spec information for chromatographic profile peaks

	*Tentative ID	RT	$[M+H]^+$	MS^2 fragments
1	chlorogenic acid	9.9	355	163
2		10.1	579	427,409,301,291,247,165
3	catechin	10.5	291	165,151,139,123
4	rutin	10.9	611	465,303
5		11.4	575	413,396,395,381,335,226,188
	7-hydroxymitragynine	11.9	415, 433	397,383,299,240,238,226,190,168
6	corynoxine	12.7	385	353,269,267,160
7		13.1	415	383,351,299,297,271,226,190
8	corynantheidine	13.5	369	337,238,226,194,174
9	mitragynine	13.9	399	367,328,238,226,174
10	speciogynine	14.1	399	367,328,238,226,174
11	speciociliatine	14.3	399	367,328,238,226,174
12	paynantheine	14.9	397	365,309,281,265,227,186,160

Figure 1. Chromatographic Profile for Kratom Extract

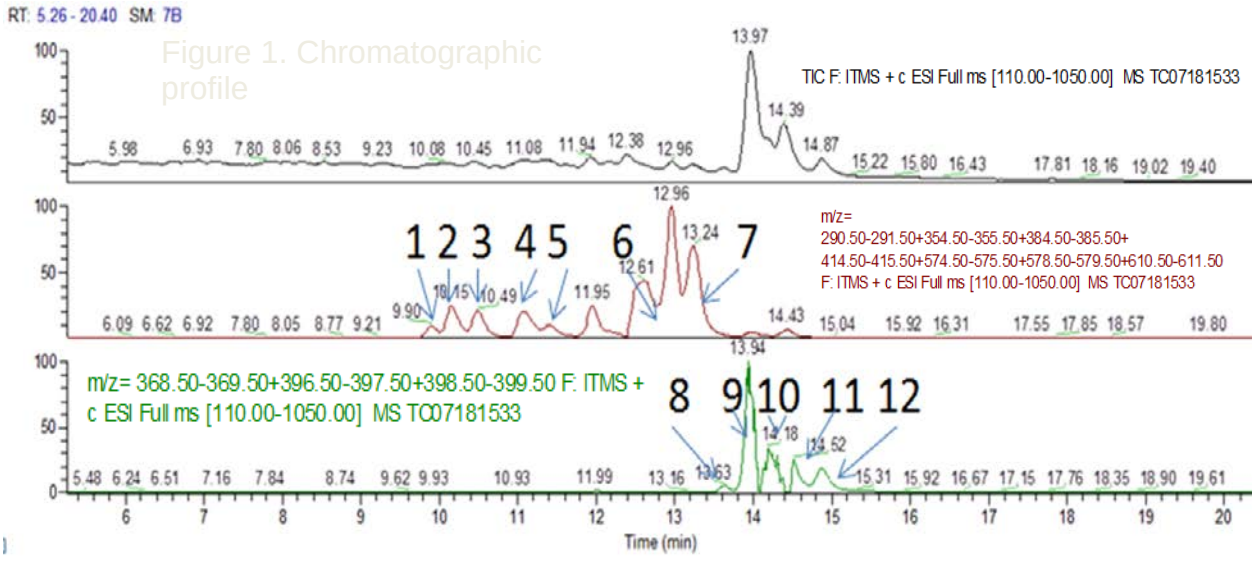

Table 2. Mitragynine content in regulatory samples.				
Bulk Powders and leaf materials				
Declared Product Identity	**matrix**	**mg/g**	**Mitragynine spikes**	
Dried Leaf (Mitragyna speciosa) for herbal incense	dried leaf	7.9		103, 109
Kratom - Bali Powder	powder	18.8		95, 94
Mitragyna speciosa	ground leaf	17.8		100, 98
Kratom	powder	12.7		95, 100
Mitragyna speciosa (Kratom) Maeng Da	powder	16.1		---
Mitragyna speciosa (Kratom) White Borneo	powder	14		96, 93
Natural Textile Coloring 1	powder	16		---
Natural Textile Coloring 2	powder	8.7		---
Natural Textile Coloring 3	powder	15.2		---
Natural Textile Coloring 4	powder	16.7		---
Natural Textile Coloring 5	powder	16.5		---
Natural Textile Coloring 6	powder	7.7		---
Natural Textile Coloring 7	powder	13.6		---
Scrubs of soap material	powder	17.3		127, 145
Agarbatti Dhoop powder, room freshener	powder	7.4		92, 93
Dosage Form Products				
Declared Product Identity	**matrix**	**mg/g**	**mg/dose**	**Mitragynine spikes**
Kratom Capsules	capsule	10.8	5.1	92, 105
Kratom Capsules	capsule	17	7	83, 72
Kratom - Thai Capsules	capsule	10.9	6	93, 84
Kratom - Maeng Da Capsules	capsule	18.3	9	101, 113
Kratom - Bali Capsules	capsule	18.8	9.3	102, 116
Vietman Kratom Capsules	capsule	18.2	9.2	75, 76
Green Tea Capsules	capsule	0.77	0.4	---
Unknown tan capsules	capsule	0.8	0.4	101, 103
Declared Product Identity	**matrix**	**mg/mL**	**mg/dose**	
Herbal Dietary Supplement Drink	liquid	0.7	41.3	94, 103

CONCLUSIONS

An LC/MS method is used to determine the mitragynine content and to confirm the identity of regulatory samples as *Mitragyna speciosa* (Kratom). The method has been successfully applied to a variety of sample matrices and is routinely used for the analysis of regulatory samples. Mitragynine linearity is >0.999, LOD is 15 ppm and 150 ppm for 7-OH-mitragynine and mitragynine, respectively. Spike recoveries for mitragynine ranged from 72-145%. Reproducibility was <4%.

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