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Chemical analysis of an herbal opioid

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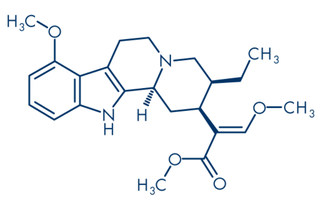
Kratom

- Kratom is an all-natural dietary supplement traditionally used to increase energy, stamina, and limit fatigue[1]
- Uses of kratom have expanded to include feelings of euphoria, sedation, and decreased pain for short term usage at high dosages[2]
- Effects of long-term use include anorexia, weight loss, insomnia, and in some more extreme cases hallucinations. [2]
- *Mitragynine* is the component that interacts with opioid receptors in the brain[3]
- Kratom possesses the potential to become adductive to its users.[4]



Mitragynine
Molecular weight: 398.50 g/mol
Molecular formula: $C_{23}H_{30}N_2O_4$

Experimental procedures

Ultrasonification extraction

0.5 g sample extracted with 10 ml of 80% methanol, sonicated for an hour, stored overnight, centrifuged for 5 minutes, and then filtered twice.

Expresso Extraction

4.5 g sample purged with 200 mL 60% methanol, stainless steel capsule, used borosilicate filter on top, 100 mL 60% methanol was used to extract contents of sample, two methods were used; a single run of ~50 mL of methanol or three runs of ~50 mL of methanol through the same cap, replacing borosilicate filter before each run.

Standard Preparation

Analytical standard of *mitragynine* was purchased and diluted to 100 ml standard with 900 µl methanol.

Serial Dilution Procedure

Concentrations of standard were 20 µg/mL, 40 µg/mL, and 60 µg/mL to a final volume of 1.00 mL. Each of the three concentrations then underwent a serial dilution to create a calibration curve.

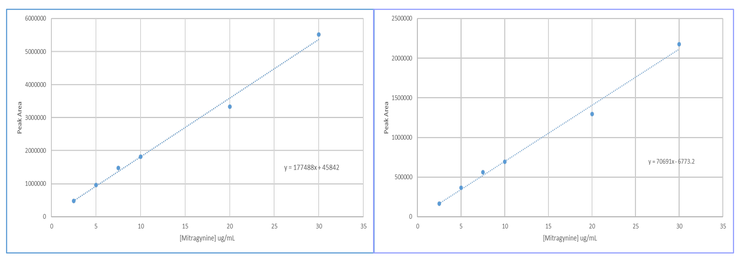


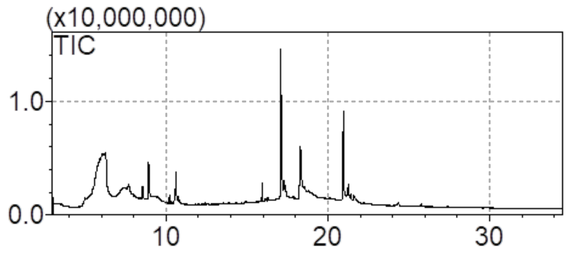
Figure 1: *Calibration Curve*

Figure 1, a, calibration curve using HPLC- Fluorescence Detector, Figure 1, b, calibration curve using HPLC- PDA detector

Gas Chromatography Mass Spectrometry

For the GCMS analysis ultrasonification extraction methods were used. This methodology can be lengthy but is used for the replicability of an analysis. ultrasonification methods follow four basic steps, (1) the solvent penetrates the solid matrix; (2) the solute dissolves in the solvents; (3) the solute is diffused out of the solid matrix; (4) the extracted solutes are collected. [5] Through ultrasonification methods a qualitative analysis using GCMS can be preformed on both the amazon sample and the kratom sample to determine the presence of mitragynine.

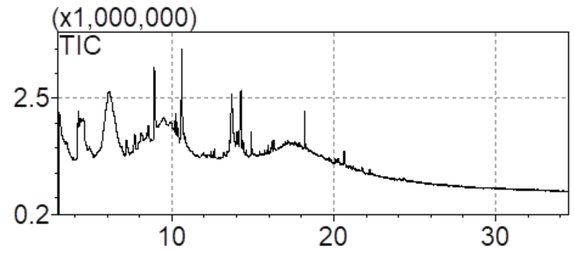
Gold Bali, Kratom Sample



Key Components

Rhynenophylline, Specofoline, Mitragynine, Squalene, 9,12,15-octadecatrienoic acid, Quinic acid, n-hexadecenoic acid

Amazon 'Knock off' Kratom Substitute



Key Components

Alpha- Amyrin, Alpha-tocopherol acetate, Hexasoloxane, Trisiloxane, Butylated hydroxyanisole, Isosorbide, 8,11,14-Eicosatrienoic acid

High Performance Liquid Chromatography

This method for extraction offers a fast, effective, and quantitative tool for the extraction of bioactive compounds from food samples with an extraction time lower than 30 seconds, using global available and low-cost equipment.[6]

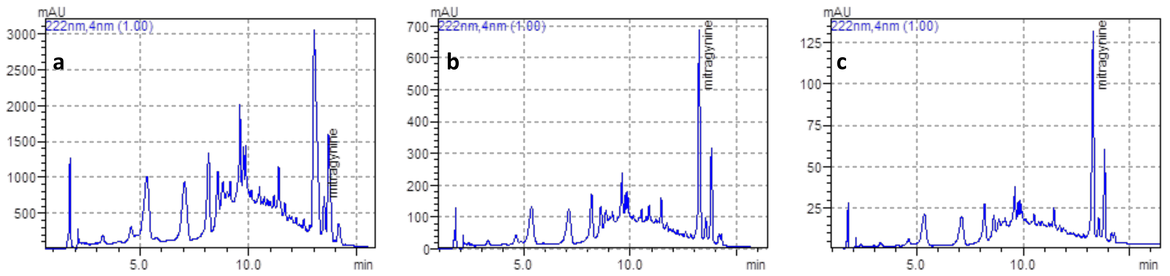


Figure 2: *Extraction method comparison of Gold Bali Kratom sample*

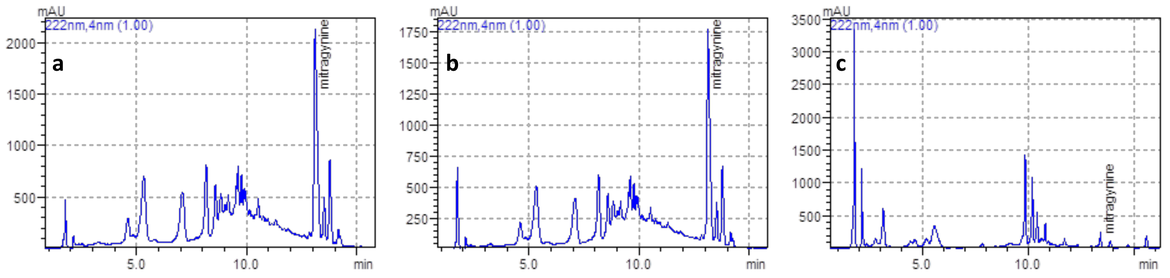


Figure 3: *Mitragynine concentration comparison between various samples*

HPLC data shows the comparison between the extraction methods for the Gold Bali Kratom sample. Figure 2, a shows the peak height of mitragynine for each extraction method used, The ultrasonification extraction method is shown in Figure 2, a, the Expresso extraction with three runs is shown in Figure 2, b, and the expresso method with one run is shown in Figure 2, c.

Samples were prepared and mitragynine was extracted using the same ultrasonification method and the peak areas of mitragynine were recorded. Figure 3, a, is the White Dragon Kratom sample, and it has a peak area of $2.5x10^7$. Figure 3, b, is the Green Malaysian Kratom sample, peak area of $2.3x10^7$. Lastly, Figure 3, c is the Amazon "Knock-off", with a peak area of $1.86x10^6$.

	[mitragynine] through different extraction tecniques		
Sample	*Ultrasonification*	*Expresso x3 Runs*	*Expresso x1 Run*
Gold Bali	82.221	73.512	43.075 ± 36.7
White Dragon	359.840	160.290	-
Red Dragon	246.244	104.009	-
Green Malasyan	323.292	76.754	-
Amazon "Knock-off"	26.452	-	2.197

Table 1: *Mitragynine Concentration*

Concentration of mitragynine throughout various samples for each extraction technique.

Instrumentation

HPLC-
Shimadzu prominence HPLC system with; diode array detector at 222 nm also; fluorescence detector with excitation at 280 nm and emission at 320 nm
GCMS-
GC- 2010 plus Shinadzu with a quadrapole mass spectrometer detector

References

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Acknowledgements

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