Stability of Mitragynine and 7-Hydroxymitragynine

Stephanie Basiliere, BS* and Sarah Kerrigan, PhD

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77341

ABSTRACT

Mitragynine (MG) and 7-hydroxymitragynine (MG-OH) are pharmacologically active alkaloids found in kratom, a natural product derived from *Mitragyna* speciosa. Hospitalizations and fatalities involving the drug have been reported due to its growing popularity as a recreational drug and for the non-medically supervised treatment of opioid abstinence syndrome. The chemical stability of these compounds has not been thoroughly described. In this report, the pH and temperature-dependent stability of mitragynine (MG), and 7-hydroxymitragynine (MG-OH) were investigated using accelerated conditions. Short-term stability was determined over a range of pH (2-10) and temperature (4-80°C) over 8 hours. Liquid chromatography time-of-flight mass spectrometry (LC-Q/TOF-MS) was used to estimate half-lives and identify degradation products where possible. MG-OH was the least stable alkaloid, with significant drug loss at 8 hours when exposed to temperatures of 40°C and above. No significant drug losses were observed for MG in aqueous solution (pH 2-10) at 4, 20 or 40°C. Two degradation products of mitragynine under basic and acidic conditions were identified.

INTRODUCTION

Kratom is a psychoactive botanical drug that can be difficult to analyze due to the presence of numerous structurally related alkaloids and isomers (1). Mitragynine is the principal alkaloid in many kratom preparations. 7-Hydroxymitragynine, which is also present in the plant, is a confirmed metabolite of mitragynine (Figure 1). Notably however, the metabolite has increased potency relative to the parent drug. Its affinity for the mu-opioid receptor is ten-fold that of morphine. Information regarding the stability of mitragynine and 7-hydroxymitragynine is relatively limited, but studies have reported that MG and MG-OH are unstable in simulated gastric fluid (pH 1.2) but stable in simulated intestinal fluid (pH 6.8) for 2-2.5 h (2). An improved understanding of the stability of these alkaloids can provide insight into information concerning bioavailability, absorption, and analytical, and pre-analytical factors, such as storage and specimen integrity.

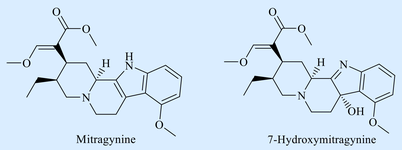

Figure 1. Chemical structure of mitragynine and 7-hydroxymitragynine.

RESULTS & DISCUSSION

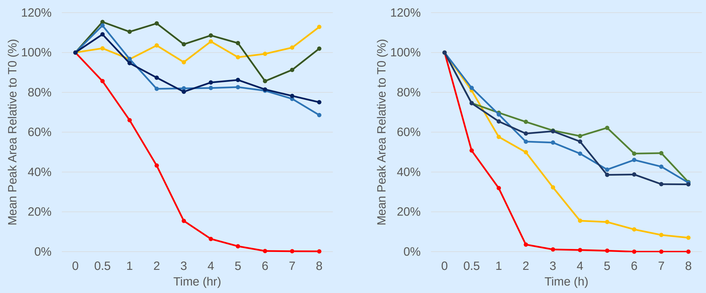

Figure 2. Representative stability plots of mitragynine and 7-hydroxymitragynine (80ºC).

Analyte	pH	Half-Life (h)	
		80°C	60°C
MG	2	0.8	9.5
	8	15	-
	10	18	-
MG-OH	2	0.6	2.1
	4	2.0	12
	6	7.5	12
	8	6.8	14
	10	5.6	9.2

Table 1. Half-lives for MG and MG-OH. Half-lives were only generated when instability (>20% loss of abundance) was observed

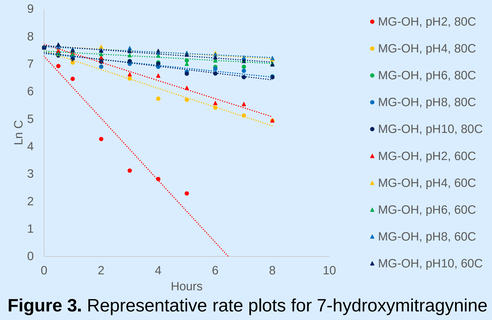

Figure 3. Representative rate plots for 7-hydroxymitragynine

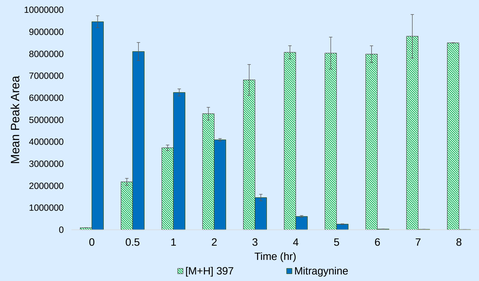

Figure 4. Formation of acidic degradation product (*m/z* 397) of mitragynine under acidic conditions.

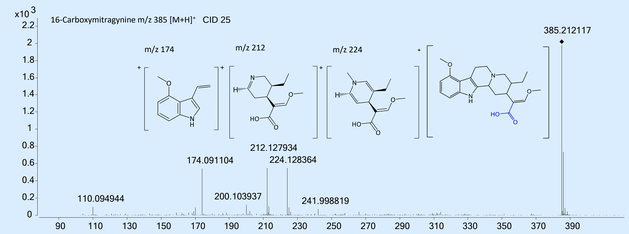

Figure 5. MS/MS spectrum of 16-carboxymitragynine

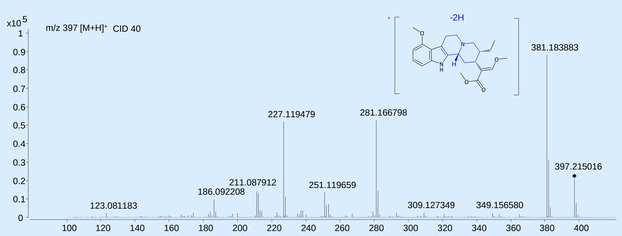

Figure 6. MS/MS spectrum of the *m/z* 397 degradation product.

Instability was observed under strongly acidic (pH 2) and basic conditions (pH≥ 8), particularly at elevated temperatures (60°C and 80°C). Overall, both alkaloids were stable at moderate conditions (~pH 6). Mitragynine and 7-hydroxymitragynine were stable at all conditions when refrigerated (≤ 4°C) or at moderate temperatures (20°C and 40°C) for at least 8 hours. The half-life for mitragynine ranged from 0.8-18h (80ºC) and 9.5h at 60ºC. Results showed that MG was more acid-labile than alkaline labile. MG was stable at pH 4 and 6, even when exposed to elevated temperatures (>60ºC). 7-Hydroxymitragynine was less stable than mitragynine and degraded significantly over the entire pH range at 60°C and above. MG-OH stability was also significantly pH dependent with half-lives ranging from 0.6 to 7.5h (80°C) and 2.1 to 14h (60°C). Two degradation products were observed for mitragynine, corresponding with $[M+H]^+$ *m/z* 385 and 397. The degradation product with m/z 385 was identified as 16-carboxymitragynine. This was produced only at alkaline conditions and was consistent with the alkaline hydrolysis of the methyl ester. The degradation product corresponding with the *m/z* 397 precursor ion was observed at acidic conditions and might be attributed to dehydrogenated mitragynine (-2 Da) or a rearrangement product, but complete structural elucidation was not possible.

Conclusions

An accelerated stability study in aqueous solution was used to investigate the temperature and pH-dependent stability of mitragynine and 7-hydroxymitragynine. Although stable at moderate pH and low temperatures, instability was observed at extreme pH. Both alkaloids were acid-labile especially when exposed to elevated temperatures. Additional studies to evaluate the long-term stability of *Mitragyna* alkaloids in biological samples are needed.

MATERIALS AND METHODS

The stability of mitragynine and 7-hydroxymitragyine was evaluated using liquid chromatography-quadrupole/time of flight-mass spectrometry (LC-Q/TOF-MS). Samples containing dilute acid (pH 2) or aqueous buffer (pH 4, 6, 8, 10) were fortified with mitragynine or 7-hydroxymitragynine to achieve a final concentration of 2,000 ng/mL. Samples were maintained at 4°C, 20°C, 40°C, 60°C and 80°C over a period of eight hours. Immediately following fortification, aliquots (50 µL, n=2) of each sample were removed and analyzed to establish T_0 (0% loss). Sampling intervals following T_0 were 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hours. Decreases in concentration were monitored using a previously validated LC-Q/TOF-MS method using targeted acquisition (3). Half-lives for each species under various conditions were estimated from rate plots. Samples were also analyzed using full scan acquisition in order to identify degradation products.

References

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