Fragmentation Pathways and Structural Characterization of Mitragynine and its Metabolite using Electrospray Ionization and High Resolution Mass Spectrometry

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ABSTRACT

Mitragynine (9-methoxycorynantheidine, Kratom, MG) is a naturally occurring corynanthe-type indole alkaloid that is present in the leaves of *Mitragyna speciosa*. While 7-hydroxymitragynine (MG-OH) is a minor constituent, it is considerably more potent. During the development of an analytical method to determine MG and MG-OH in urine using electrospray ionization (ESI) and liquid chromatography-quadrupole/time of flight mass spectrometry (LC-Q/TOF-MS), the fragmentation pathways for MG, MG-OH and their deuterated analogs were investigated. MS-MS spectra were used to tentatively identify fragments and make mass assignments. A total of four transitions were selected for each of the compounds (and their respective internal standards). The most abundant product ions for both MG and MG-OH were associated with C-ring cleavage and the loss of the substituted piperidine (D-ring) between C2 and C5. The abundance and specificity of these transitions ultimately led to their selection for quantitation of both MG (399→174) and MG-OH (415→190). Variations of C ring cleavage predominated for all other major product ions, as well as formation of intact substituted piperidine ions.

INTRODUCTION

Kratom is a psychoactive drug that comes from the leaves of the Korth (*Mitragyna speciosa*) tree. Kratom usage was first recorded in the early twentieth century and it is a known herbal drug in Southeast Asia (1). Kratom is a powerful psychoactive drug that can produce anorexia, hallucinations and delusional behavior (2). Kratom's dominant alkaloid is mitragynine (MG), but it also contains a number of other alkaloids, including 7-hydroxymitragynine (MG-OH). MG an MG-OH are both μ-opioid receptor agonists (2).

Kratom is widely available for sale on the internet and through retail outlets. Recreational use of Kratom has increased in Western Europe and the United States (3). Kratom is not currently scheduled in the US and although Kratom usage has been reported for over 100 years, additional research is needed to better understand this drug.

Identification of MG and MG-OH in biological samples presents a significant challenge in terms of analytical detection. LC-Q/TOF-MS is a high resolution MS technique that offers high sensitivity and significant benefits in terms of mass accuracy and structural identification. Identifying fragmentation pathways and subsequent structural identification of ions is an important role of method development. In this study, during the development of an analytical method for these compounds in urine using LC-Q/TOF-MS, we identified the fragmentation pathways of MG and MG-OH.

MATERIALS AND METHODS

Chemicals

Reference standards for mitragynine, 7-hydroxymitragynine, mitragynine-D3, and 7-hydroxymitragynine-D3 were purchased at a concentration of 100 μg/ml from Cerilliant (Round Rock, TX).

Sample Preparation

Mixed mode solid phase extraction (SPE) was used to extract mitragynine and 7-hydroxymitragynine from urine samples. Drug-free urine (1 mL) was fortified with various concentrations of the mitragynine and 7-hydroxymitragynine working standards (5 ng/mL). To each tube, 50 μL (2 ng/μL) of the working standard containing mitragynine-D3 and 7-hydroxymitragynine-D3 were added. Samples were then fortified with 2 mL of 0.1M HCl solution and added to the SPE columns. The columns were then washed with 1 mL of deionized water and 1 mL of 1 M acetic acid. The samples were dried for 5 minutes and then washed with hexane (1 mL), ethyl acetate (1 mL), and methanol (1 mL). MG and MG-OH were eluted using 1 mL of ethyl acetate containing 2% ammonium hydroxide and were then reconstituted in 30 μL of 50/50 (v/v) mobile phase A:B solution composed of DiH_20 with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The reconstituted samples were transferred to LC autosampler vials.

Instrumentation

Analysis was achieved using an Agilent 6530 Accurate Mass LC-Q/TOF-MS with a heated electrospray ionization (ESI) source. Chromatography was performed with an Agilent Poroshell EC-C18 Column (2.1 x 100 mm, 2.7 μm particle size) and an Agilent Poroshell 120 EC-C18 Guard Column (2.1 x 5 mm, 2.7 μm particle size). The method used multiple reaction monitoring mode (MRM) using a minimum of 4 transitions (3 qualifier ions and a quantitation ion for each compound). All data analysis was performed using Agilent MassHunter software.

RESULTS

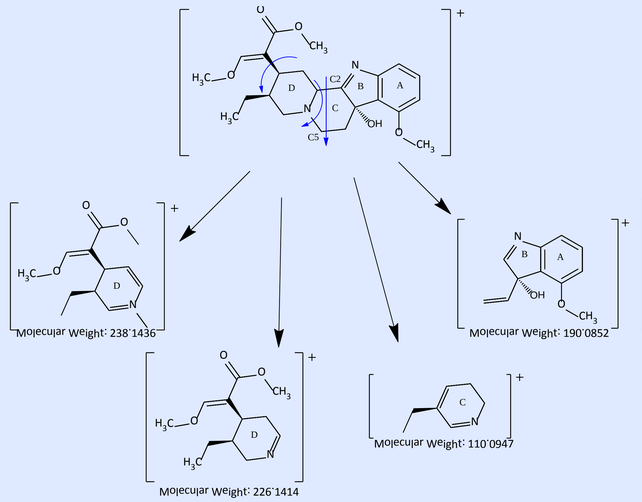

Figure 1: 7-Hydroxymitragynine

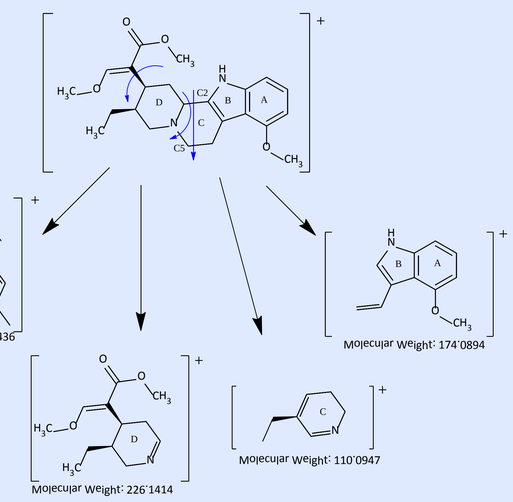

Figure 2: Mitragynine

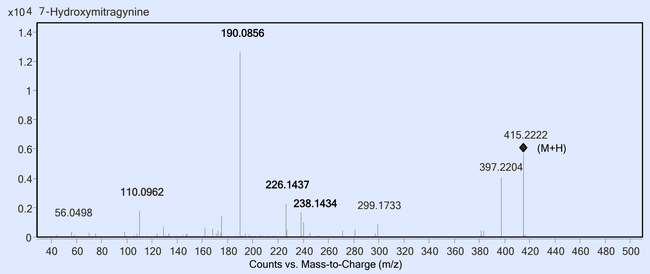

Figure 3:7-Hydroxymitragynine Mass Spectrum

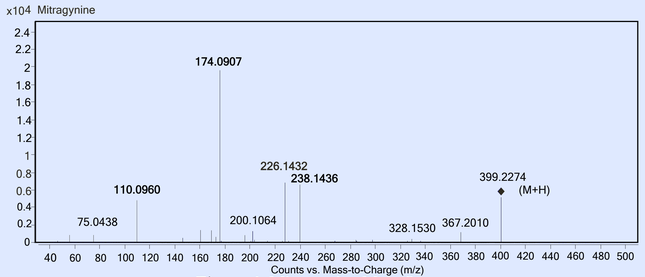

Figure 4: Mitragynine Mass Spectrum

DISCUSSION & CONCLUSIONS

Method development for the compounds in *M. speciosa* is challenging due to the large number of structurally similar alkaloids and diastereoisomers found in the plant. LC-Q/TOF-MS and other high resolution MS techniques are particularly useful for complex analytes such as these. In addition, methods utilized during the analysis of forensic toxicological samples must be highly sensitive and selective. Identifying the fragmentation pathways can improve the selectivity of a method because it allows for highly specific precursor ion transitions to be used for identification and quantitation.

The MS/MS spectra for mitragynine and 7-hydroxymitragynine (**Figures 1 and 2**) were used to tentatively identify fragments and determine fragmentation pathways. A total of four transitions for each compound were selected from the MS/MS spectrums (**Figures 1 and 2**) for this analytical method. The transitions 399→238, 399→226, 399→174, and 399→110 were selected for mitragynine and 415→238, 415→226, 415→190, and 415→110 for 7-hydroxymitragynine.

The proposed fragmentation pathways are shown in **Figures 3 and 4**. The most abundant product ions for mitragynine and 7-hydroxymitragynine (174 and 190, respectively) were associated with C-ring cleavage and the loss of the substituted piperidine (D-ring) between C2 and C5. The abundance and specificity of these transitions ultimately led to their selection for quantitation purposes. Variations of C-ring cleavage and the formation of intact substituted piperidine ions predominated for all other major product ions that were later chosen as qualifier transitions (238, 226, and 110, respectively).

REFERENCES

1. Hassan, Z., Muzaimi, M., Navaratnam, V., Yusoff, N.H.M., Suhaimi, F.W., Vadivelu, R., Vicknasingam, B.K., Amato, D., von Horsten, S., Ismail, N.I.W., Jayabalan, N., Hazim, A.I., Mansor, S.M., Muller, C.P., 2013. From Kratom to mitragynine and its derivatives: Physiological and behavioural effects related to use, abuse, and addiction. Neuroscience and Biobehavioral Reviews 37, 138-151.

2. Horie, S., Yamamoto, L.T., Moriyama, T., Yano, S., Takayama, H., Aimi, N., Sakai, S., Ponglux, D., Shan, J., Pang, P.K.T., Watanabe, K., 1998. Pharmacological characteristics of mitragynine, an indole alkaloid from Thai medicinal herb, as an opioid receptor agonist. Naunyn-Schmiedeberg's Archives of Pharmacology 358, R70-R70.

3. Drug Enforcement Administration, January 2013. KRATOM (*Mitragyna speciosa korth*) http://www.deadiversion.usdoj.gov/drug_chem_info/kratom.pdf.

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