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A Mass Spectrometric Study of Kratom Compounds by Direct Infusion Electrospray Ionization Triple Quadrupole Mass Spectrometry

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Abstract

Mitragynine (MG) and its major metabolites 7-hydroxymitragynine (7-OH-MG) are two of the major components of the plant extract Kratom, which is a tree planted in Southeast Asia. Kratom has long been used by opioid-dependent individuals as an alternative to their unavailable opioid of choice and chronic pain medication, as a stealth-to-urine drug screening opiate substitute while in opioid recovery treatment and recreationally, alone or as a booster. In this study, a direct infusion method was utilized and electrospray ionization triple quadrupole mass spectrometer was used as the detector for data acquisition. Pharmacokinetic study was conducted to investigate the effect of mitragynine and 7-hydroxymitragynine and major fragments of both compounds were proposed.

Keywords

Mitragynine, 7-Hydroxymitragynine, Kratom, HPLC-MS/MS, Pharmacokinetic, Mass Fragment

1. Introduction

Mitragynine (MG) and its major metabolites 7-hydroxymitragynine (7-OH-MG) are two of the major components of the plant extract Kratom [1] [2]. Kratom is derived from the *Mitragyna speciose* Korth tree in Southeast Asia, and like oxycodone, it has rare paradoxical properties consisting of augmented focus, concentration and physical energy, with concurrent reduction in physical and emotional pain [3] [4]. In the 1950s, mitragynine and its metabolites were prohibited by law in Thailand and in 2003 in Malaysia [5]-[8]. The U.S. Drug Enforcement

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Administration (USDEA) Office of Diversion Control lists kratom as a drug of concern; however, kratom remains legal in the U.S. and is also one of the most popular legal highs in the U.K. [9] [10]. *M. speciose* Korth contains more than 25 alkaloids that vary quantitatively depending on geographic location.

Several unique alkaloids are present in kratom leaves and the predominant alkaloid is mitragynine. Mitragynine (**Figure 1**) is structurally similar to the aphrodisiac yohimbine, which is the most prevalent of these alkaloids, and mitragynine is believed to be responsible for kratom's opioid effects [11] [12]. Another less prevalent alkaloid of kratom, 7-hydroxymitragynine, has also been studied for different functionality such as analgesic activity. 7-hydroxymitragynine is orally active in animals as an analgesic, and produces normal opioid side effects including constipation, with similar chemical structure to mitragynine compound.

In recent years, the hyphenated technique of chromatography and electrophoresis coupling with mass spectrometry has been widely used for different applications, for instance, kinetic study of cylindrospermopsin under TiO_2 photocatalytic reaction [13], treatment of 6-hydroxymethyl uracil [14], *Microcystis aeruginosa* and microcystincyanotoxin [15], chiral separation of cathinone analogs [16] [17], microcystins separation and detection [18], and fluorescent substrate for monitoring acid phosphatase activity [19] [20].

In the previous study, it was focused on the method development and validation using LC-MS/MS [21]. A rapid and effective method for quantification of mitragynine and 7-hydroxymitragynine compounds in human urine matrix was reported. Correlation coefficients greater than 0.99 were obtained for both mitragynine and 7-hydroxymitragynine compounds in the previous study [21]. In the current study, mass spectrometry part was emphasized with mass spectrum and mass fragments information. Pharmacokinetic and metabolites were examined using direct infusion electrospray ionization technique.

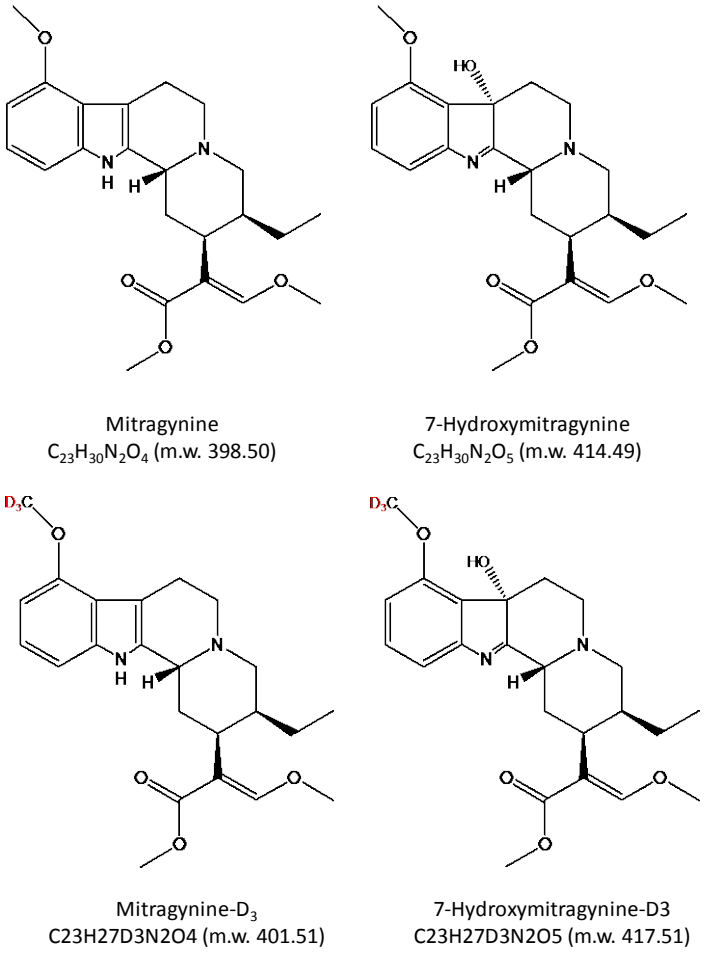

Figure 1. Chemical structures of mitragynine, mitragynine-D_3, 7-hydroxymitragynine, and 7-hydroxy mitragynine-D_3.

2. Experimental

2.1. Reagents

Acetonitrile and HPLC-grade water are purchased from EMD Millipore (Billerica, MA, USA). Formic acid was purchased from Amresco (Solon, OH, USA). Mitragynine with a concentration of 100 µg/mL and 7-hydroxymitragynine with a concentration of 100 µg/mL standards were purchased from Cerilliant (Round Rock, TX, USA). Internal standards mitragynine-D_3 with a concentration of 100 µg/mL and 7-hydroxymitragynine-D_3 with a concentration of 100 µg/mL were purchased from Cerilliant (Round Rock, TX, USA). Internal standards were used for quantification purposes as a mean for correct the loss of analytes of interest during sample preparation process or sample injection. The phenyl-hexyl HPLC column was purchased from Phenomenex (Torrance, CA, USA).

2.2. LC-MS/MS Instrumentation

The assay was developed on a Shimadzu 20AD liquid chromatography (Columbia, MD, USA) coupled to an AB SciexQTrap 5500 quadrupole linear ion trap mass spectrometer (Framingham, MA, USA). A 2.6-µm 100 mm × 2.1 mm phenyl-hexyl analytical column was employed, and gradient elution with a 0.4-mL/min flow rate of water and acetonitrile as mobile phases was utilized. The LC-MS/MS conditions were optimized to achieve rapid and effective goals for the detection of kratom compounds.

2.3. Preparation of Standard Solutions

Stock solutions were prepared weekly to keep the active component fresh. Methanol standards and urine standards were prepared for injection, respectively. The methanol standards were stored at −8˚C, and the urine standards were stored at 4˚C. For HPLC injection, 50 µL of working standards, 50 µL of 10 ng/mL internal standards and 150 µL of Mobile Phase A solution were mixed as the injection standards. For testing on urine samples, working standards were substituted with human urine.

2.4. Sample Extraction

Both blank and patient urine samples were stored at −20˚C until analysis. Urine samples were thawed and 1.0 mL aliquot was transferred to a 4 mL clear glass screw-top culture tube and spiked with 50 µL of 10 ng/mL internal standards. The urine samples were centrifuged at 15,000 rpm speed for 15 min and the supernatant was transferred to a clue tube. With addition of internal standards and diluent, the mixture was transferred to an HPLC vial for HPLC-MS/MS analysis.

2.5. Calibration

Quantitation of mitragynine and 7-hydroxymitragynine were calibrated by internal standard technique. Deuterated internal standards purchased from Cerilliant (Round Rock, TX, USA) were added to the sample mixture as a calibration technique. The calibration curve was constructed by plotting the ratios of the peak area of mitragynine and mitragynine internal standard against the ratios of concentration of mitragynine and mitragynine internal standard. The 1/x regression model was employed to acquire the regression equation and coefficient (r).

3. Results and Discussion

3.1. HPLC Method Development

Analytes were eluted with gradient mobile phases of water with 0.1% formic acid (Mobile Phase A) and acetonitrile (Mobile Phase B). Formic acid is a commonly-used additive for reversed-phase liquid chromatography, as it provides protons and promotes ionization for analytes. Acetonitrile is an organic solvent that provides advantages over methanol in terms of low back pressure, high sensitivity and less ghost peak for the gradient elution program.

The separation and identification of mitragynine and 7-hydroxymitragynine are demonstrated as **Figure 2**. The extracted chromatograms of both compounds are presented. As shown in the figure, 7-hydroxymitragynine eluted faster than mitragynine with the above mentioned conditions.

3.2. MS/MS Optimization

The operating conditions and parameters for the electrospray ionization source were optimized to obtain the best mass spectrometric performance for both mitragynine and 7-hydroxymitragynine. The mass spectrometry parameters are listed as **Table 1**.

3.3. Mass Spectra

With the assistance of direct infusion electrospray ionization mass spectrometry technique, mass spectra and information such as molecular weight and mass fragment were obtained. Possible cleavage and metabolites mechanisms are proposed as **Figure 3** for mitragynine and **Figure 4** for 7-hydroxymitragynine. As indicated by the mass spectrum parameters in method development, the transition 174.3 is the major fragment of protonated mitragynine compound under instrument conditions stated in **Table 1** and **Table 2**.

As illustrated in **Figure 3**, protonated mitragynine with *m/z* 399.5 has fragment of *m/z* 174.3, 226.1 and 238.2. **Figure 4** demonstrated the major fragment of protonated 7-hydroxymitragynine *m/z* 415.5 is *m/z* 190.2.

3.4. Method Validation

The analytical figures of merit were assessed and presented in **Table 3**. Linearity in terms of slope and R-squared value was determined. Sensitivity including LOD and LOQ, intraday precision and interday precision were calculated as well. Limit of detection down to 0.0123 ng/mL for mitragynine and 0.0691 ng/mL for 7-hydroxymitragynine were achieved. The LOD values show that the established method is very sensitive for trace amount analysis.

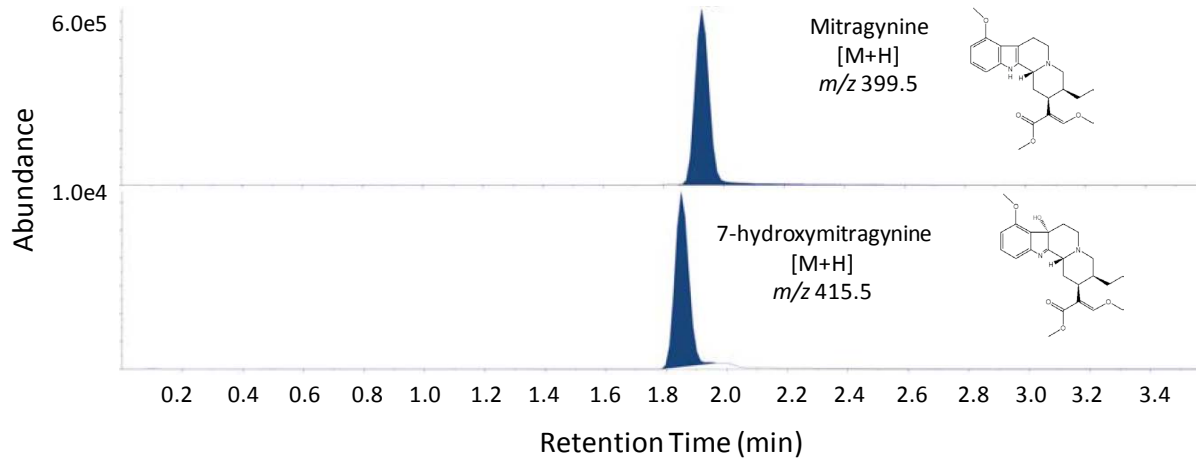

Figure 2. Extracted chromatograms of mitragynine and 7-hydroxymotragyninewith 0.1% formic acid and acetonitrile as mobile phases.

Table 1. Optimized MS/MS operating conditions for mitragynine and 7-hydroxymigragynine obtained from tandem mass spectrometry.

MS/MS conditions	Mitragynine	7-hydroxymitragynine
Polarity	Positive	Positive
Ionspray voltage	4000 V	4000 V
Temperature	550˚C	550˚C
Collision gas	Medium	Medium
Ion source gas 1	50.0	55.0
Ion source gas 2	65.0	65.0

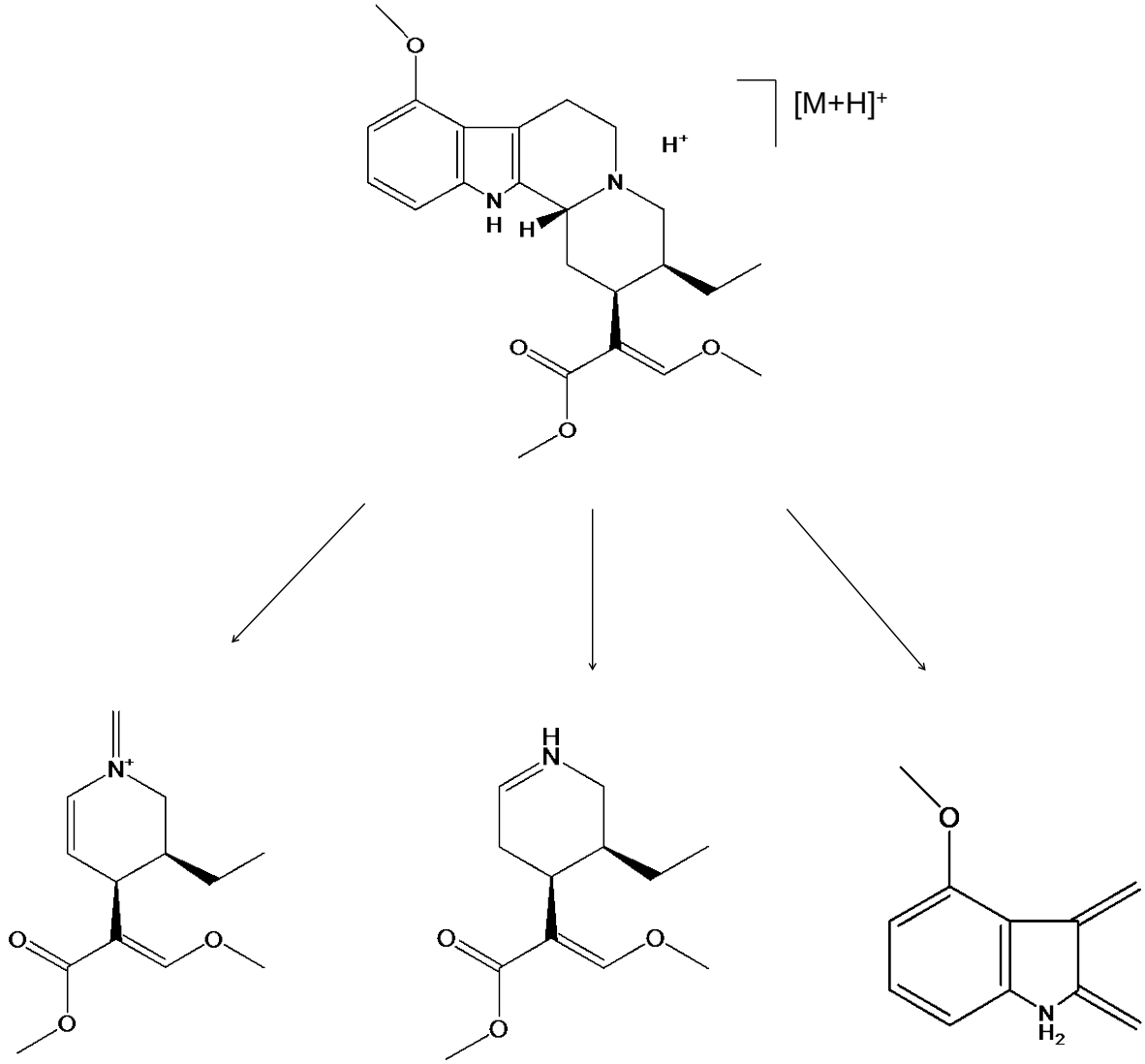

Figure 3. Mass fragmentation patterns of mitragynine.

Table 2. Multiple reaction monitoring (MRM) parameters of mitragynine and 7-hydroxymitragynine (analytes) and mitragynine-D_3 and 7-hydroxymitragynine-D_3 (internal standards).

MS/MS conditions	MG	7-OH-MG	MG-D_3	7-OH-MG-D_3
Precursor ion (*m/z*)	399.5	415.5	402.5	418.6
Product ion (*m/z*)	174.3	190.2	238.3	193.2
Collision energy (eV)	45	45	35	45

Table 3. Analytical figures of merit of LC-MS/MS results.

Analytes	Linearity		Sensitivity		Precision (intraday, n = 5)	Precision (inter-day, 3d/n = 6)
	Slope	R^2	LOD (ng/mL)	LOQ (ng/mL)	RSD%	RSD%
Mitragynine	92.5	0.9987	0.0123	0.0356	1.55	1.70
7-hydroxymitragynine	87.5	0.9951	0.0691	0.215	2.12	2.71

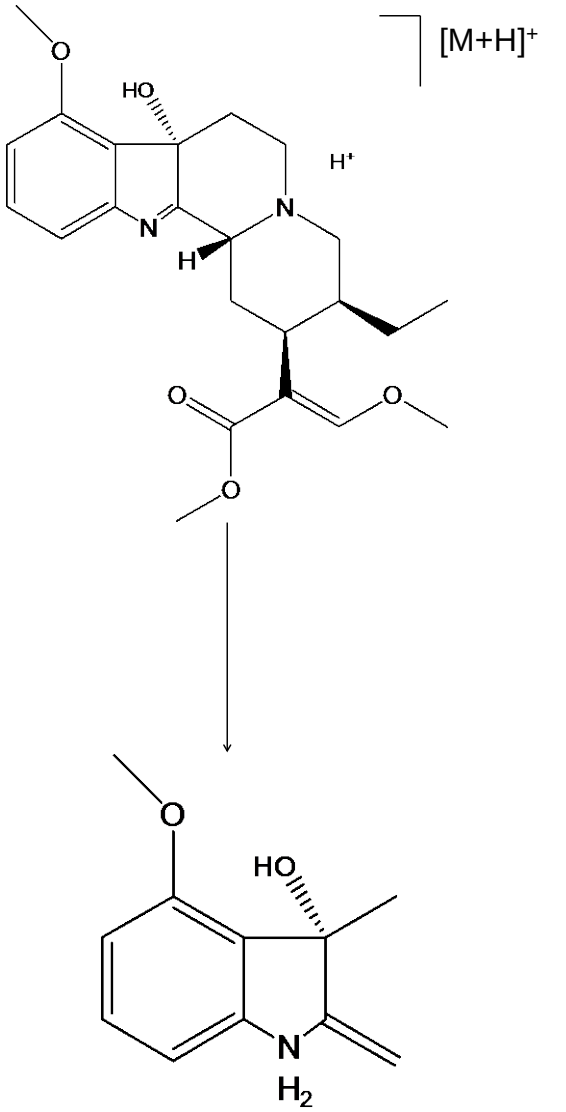

Figure 4. fragmentation patterns of 7-hydroxymitragynine.

4. Concluding Remarks

In this study, a specific detection and identification method by direct infusion electrospray ionization mass spectrometry has been developed for analysis of mitragynine and 7-hydroxymitragynine. The method demonstrates a rapid and precise route for the detection and identification in pharmaceutical and biomedical applications. This approach provides capability of mass spectrum and mass fragments information, which facilitates the pharmacokinetic study of mitragynine and 7-hydroxymitragynine compounds.

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