American Journal of Chemistry and Application
2015; 2(4): 61-65
Published online August 10, 2015 (http://www.aascit.org/journal/ajca)
ISSN: 2375-3765

AASCIT American Association for Science and Technology

Keywords
Kratom,
Chromatography,
Mass Spectrometry,
Method Development

Received: July 22, 2015
Revised: July 29, 2015
Accepted: July 30, 2015

Determination of Kratom Using High Performance Liquid Chromatography Tandem Mass Spectrometry

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Citation
Zhanglei Li. Determination of Kratom Using High Performance Liquid Chromatography Tandem Mass Spectrometry. *American Journal of Chemistry and Application.* Vol. 2, No. 4, 2015, pp. 61-65.

Abstract
Chromatography coupling to mass spectrometry becomes a vital instrumentation tool for the separation and determination of small molecules qualitatively and quantitatively. In this study, the author is focused on the mitragynine and 7- hydroxymitragynine. Mitragynine and its major metabolite, 7-hydroxymitragynine (also known as "kratom"), are drugs which extracted from a tree planted in Thailand, Myanmar and other Southeast Asian countries. Nowadays, kratom compounds are used as recreational drugs in many countries all over the world. The previous report studied a novel method for screening and identification of mitragynine and 7-hydroxymitragynine in human urine by liquid chromatography tandem mass spectrometry. However, the method conditions were not optimized. The objective of this study is to explore a rapid approach to separate and identify the kratom compounds by new instrumentation- ultra high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). Ultra high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) was utilized to perform method development and optimization. Experiments were carried out using a state-of-the-art ultra high performance liquid chromatography system. The method was found linear in the range of 10 - 100 ng/mL. In the linearity study, good regression equation and correlation coefficient were achieved. Comparing to HPLC system, UHPLC exhibited short analysis time and high selectivity. The optimized method can be used for the separation and detection of mitragynine and 7-hydroxymitragynine.

1. Introduction

In the current world, the drugs and drugs of abuse become a main issue due to the widely used internet, social media, and several of other mass communication tools. Among the different types of drugs, a novel type of so called "recreational drugs", were popular in the teenagers and young people groups. This type of drugs has relatively low toxicity comparing with traditional drugs, i.e. heroin. In addition, it is easy to synthesize or produce, thus the cost for the producer, and the "consumers", are relative inexpensive comparing to traditional drugs.

In the current study, the author focuses on new types of compounds named kratom. Kratom is a group of compounds which consist of mitragynine, 7-hydroxymitragynine, and other metabolites.

Mitragynine and its major metabolite, 7-hydroxymitragynine (also known as "Kratom"), are drugs which extracts from a tree planted in Southeast Asian countries [1].

Nowadays, kratom compounds are used as recreational drugs in many countries all over the world. Mitragynine and 7-hydroxymitragynine (physical properties shown as Table 1) is structurally similar to each other [2-3].

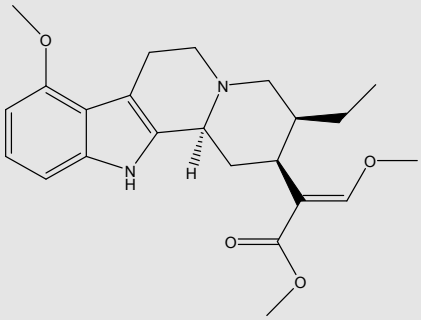
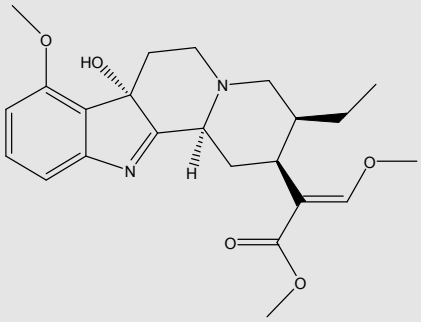
As presented in Table 1, mitragynine and 7-hydroxymitragynine have the molecular weight of 398.50 and 414.49, respectively. The chemical formula of mitragynine is $C_{23}H_{30}N_2O_4$ with Chemical Abstracts Service (CAS) registry number 6202-22-8, and the chemical formula of 7-hydroxymitragynine is $C_{23}H_{30}N_2O_5$ with CAS registry number 174418-82-7. The difference between the two compounds is only one oxygen in terms of atoms. The International Union of Pure and Applied Chemistry (IUPAC) name of the mitragynine is (E)-2-[(2S,3S)-3-ethyl-8-methoxy-1,2,3,4,6,7,12,12b- octahydroindolo [3,2-h]quinolizin-2-yl]-3- methoxyprop-2-enoic acid methyl ester, and the IUPAC name of the 7-hydroxymitragynine is (αE,2S,3S,7aS,12bS)-3-Ethyl-1,2,3,4,6,7,7a,12b-octahydro-7a-hydroxy-8-methoxy-α-(methoxymethylene)indolo[2,3-a]quinolizine-2-acetic acid methyl ester.

Chromatography is a separation technique which is widely used for various applications, including petroleum products, fast consumption products, drug analysis, amino acids, protein purifications and extractions, environmental monitoring, forensic toxicology analysis and so on.

As a new trend, chromatography coupling to mass spectrometry becomes a vital instrumentation tool for the separation and determintaiton of small molecules qualitatively and quantitatively.

To the author's knowledge, a number of scientific methods have been studied for the determination of kratom, including gas chromatography, liquid chromatography, capillary electrophoresis, and different techniques coupling to mass spectrometry [3-6] and other technique methods [7-10]. Fu *et al.* [3] reported a novel method for screening and identification of mitragynine and 7-hydroxymitragynine in human urine by liquid chromatography tandem mass spectrometry. However, the method conditions were not optimized. The objective of this study is to explore a rapid approach to separate and identify the kratom compounds by new instrumentation- ultra high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS).

Table 1. *Physical properties of mitragynine and 7-hydroxymitragynine.*

Parameters	**Mitragynine**	**7-Hydroxymitragynine**
Chemical formula	$C_{23}H_{30}N_2O_4$	$C_{23}H_{30}N_2O_5$
Molecular weight	398.50	414.49
Chemical structure		
IUPAC name	(E)-2-[(2S,3S)-3-ethyl-8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[3,2-h]quinolizin-2-yl]-3-methoxyprop-2-enoic acid methyl ester	(αE,2S,3S,7aS,12bS)-3-Ethyl-1,2,3,4,6,7,7a,12b-octahydro-7a-hydroxy-8-methoxy-α-(methoxymethylene)indolo[2,3-a]quinolizine-2-acetic acid methyl ester
CAS Registry Number	6202-22-8	174418-82-7

2. Materials and Methods

Acetonitrile and LC-MS grade water were purchased from Guangxi Chemical Company (Nanning, China). Mitragynine and 7-hydroxymitragynine standards were purchased from Shanghai Fine Chemical Company (Shanghai, China). The C-18 column was purchased from Agilent China Ltd, Co. (Shanghai, China). Calibration standard solutions were prepared prior to method development and stored at -4°C to keep the active component effective.

The experiments were carried on an Agilent 1290 Infinity LC Systems (Shanghai, China) coupling to a Sciex API 4000 triple quadrupole mass spectrometer (Shanghai, China). A 2.6-μm 50 mm × 2.1 mm C-18 analytical column was utilized with a 0.6-mL/min flow rate of mobile phases.

The mass spectrometer parameters are shown as Table 2. As presented, both mitragynine and 7-hydroxymitragynine compounds are detected under electrospray ionization (ESI) mode using positive source (ESI+). This is due to the compounds properties which [M+1] ions are easily to form during the ionization process.

The precursor ions of mitragynine and 7-hydroxymitragynine compounds are 399.3 and 415.3, respectively. The numbers match the molecular weight plus one hydrogen, which is [M+1] peak. The production ions, which also named as MRM transition ions, are 159.2 and 190.0 for mitragynine and 7-hydroxymitragynine,

respectively. The ionization spray voltage for both mitragynine and 7-hydroxymitragynine compounds are 4000 V, and the ionization temperature for both mitragynine and 7-hydroxymitragynine compounds are 600 °C. The curtain gas for the mitragynine compound is 30, and for the 7-hydroxymitragynine is 30. The most important parameter in the MRM transition, collision energy (CE), are 65 for the mitragynine, and 60 for the 7-hydroxymitragynine, respectively.

In addition to the above mentioned parameters and the parameters listed in the date table 2, a number of other parameters, such the column lengths, column diameters, column types, the LC oven, the LC mobile phases, the flow rate, the gradient program, etc. were examined as well. The optimized outcome was illustrated as Fig. 1 Extracted Ion Chromatogram of mitragynine and 7-hydroxymitragynine.

Table 2. *Parameters of mass spectrometry determination.*

Parameter	Mitragynine	7-Hydroxymitragynine
Source	ESI	ESI
Mode	Positive	Positive
Precursor Ion	399.3	415.3
Product Ion	159.2	190.0
Ion Spray Voltage	4000 V	4000 V
Temperature	600 °C	600 °C
Curtain Gas	30	30
Collision Energy	65	60

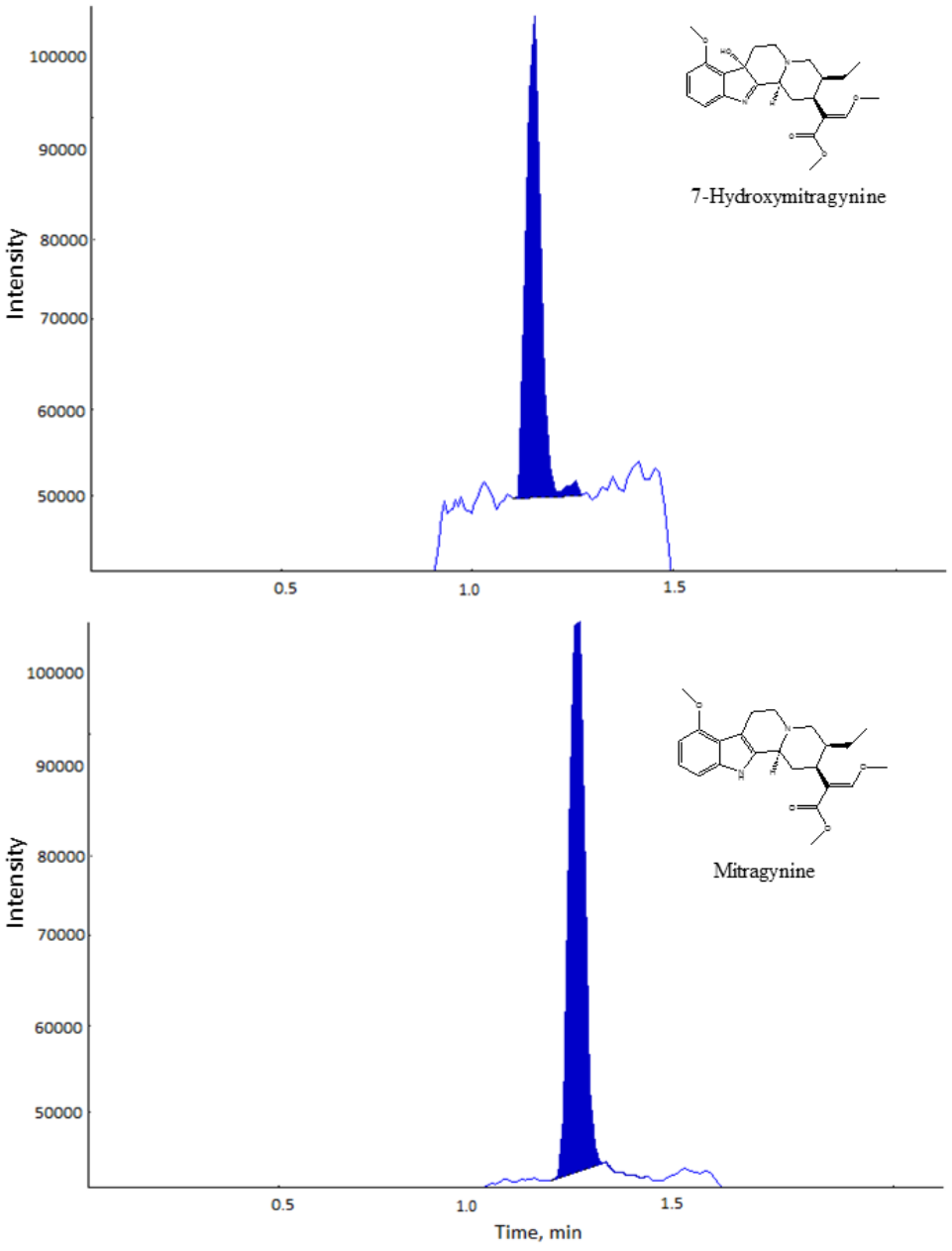


Fig. 1. *Extracted Ion Chromatogram of mitragynine and 7-hydroxymitragynine. The 7-hydroxymitragynine was eluted first at 1.26 min. The mitragynine was then eluted at 1.38 min. Mobile phases of water with 0.01% formic acid as mobile phase A and acetonitrile as mobile phase B were employed to achieve the optimized separation conditions.*

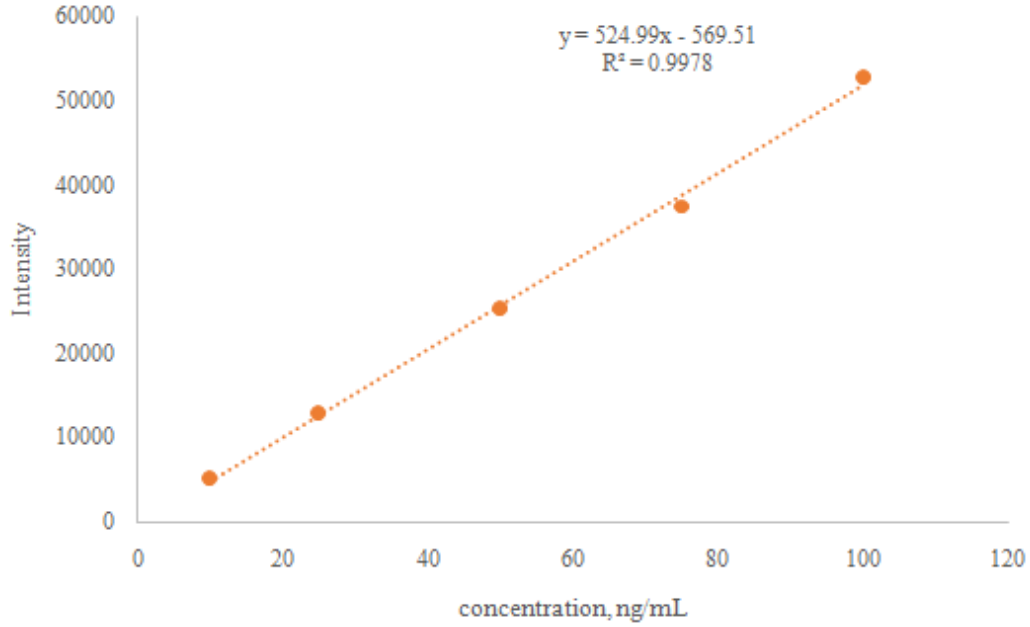


Fig. 2. *Linearity of mitragynine. Five standards at different concentrations were utilized to examine the linearity of the established method. The concentrations range from 10 ng/mL, 25 ng/mL, 50 ng/mL, 75 ng/mL, and 100 ng/mL. Three replicates were used to avoid error and the average intensity was utilized for the final calculation.*

3. Results and Discussion

Kratom compounds were eluted with mobile phases of water with 0.01% formic acid as mobile phase A and acetonitrile as mobile phase B. Fig. 1 illustrates the optimized separation results of the two analytes, mitragynine and 7-hydroxymitragynine. The gradient begins with 10% of mobile phase A, with a linear gradient to 60% within 3 min. A 3-minute post run was employed after both compounds eluted.

As demonstrated in Fig. 1, the 7-hydroxymitragynine was eluted first at 1.26 min. The mitragynine was then eluted at 1.38 min. The two compounds were separated under the previously describe conditions.

This method provides a rapid approach for separation and identification of the mitragynine and 7-hydroxymitragynine, where both compounds were eluted within 2 min.

The calibration model or linearity of the method is a mathematical representation of the correlation between instrument signal and analyte concentration [3]. To examine the linearity of the developed method, five levels of concentration were used. The peak area ratios (PAR) from these calibrators are then plotted versus concentration. After the data are plotted, they should be fitted (regressed) with a best fit line. While the R^2 or correlation value (r) may be evaluated, it is more appropriate to evaluate residuals. In general, the r should be greater than 0.98 and the residuals (back calculated values) should be within 25% of the expected values. Fig. 2 demonstrates the linearity of mitragynine.

The linearity proves that the method conditions were optimized on the instrument systems within the detection range. Generally, a 0.99 coefficient represents that a good linear range was occurred. Thus, the method can be further used for the quantitation analysis of both mitragynine and 7-hydroxymitragynine compounds.

As illustrated in Fig. 2, five standards at different concentrations were utilized to examine the linearity of the established method. The concentrations range from 10 ng/mL, 25 ng/mL, 50 ng/mL, 75 ng/mL, and 100 ng/mL. Three replicates were used to avoid error and the average intensity was utilized for the final calculation.

4. Conclusions

The current study presents a rapid and selective method for the separation and detection of mitragynine and 7-hydroxymitragynine using ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). This method can be applied for routine clinical examination, forensic toxicology case, and a number of other applications. Further method validation may be needed for the accuracy method throughout.

Acknowledgement

The author acknowledges Dr. Hanzhuo Fu for the technical support on the project.

References

[1] Jansen KLR, Prast CJ. Psychoactive properties of mitragynine (kratom). Journal of Psychoactive Drugs 1988;20(4):455-457.

[2] Assanangkornchai S, Muekthong A, Sam-angsri N, Pattanasattayawong U. The use of mitragynine speciosa ("krathom"), an addictive plant, in Thailand. Substance Use & Misuse 2007;42(14):2145-2157.

[3] Fu H, Cid FX, Dworkin N, Cocores J, Shore G. Screening and identification of mitragynine and 7-hydroxymitragynine in human urine by lc-ms/ms. Chromatography 2015;2(2):253-264.

[4] Merola G, Fu H, Tagliaro F, Macchia T, McCord BR. Chiral separation of 12 cathinone analogs by cyclodextrin-assisted capillary electrophoresis with uv and mass spectrometry detection. Electrophoresis 2014;35(21-22):3231-3241.

[5] Merola G, Fu H, Tagliaro F, Macchia T, McCord B. Chiral separation of 12 cathinone analogs by cyclodextrin-assisted capillary electrophoresis time-of-flight mass spectrometry. In the 41st International Symposium and Exhibit on High Performance Liquid Phase Separations and Related Techniques (HPLC 2014), New Orleans, LA, USA, 2014.

[6] Yang D, Li Z, Diwu YA, Fu H, Liao J, Wei C, Diwu Z. A novel fluorogenic coumarin substrate for monitoring acid phosphatase activity at low ph environment. Current chemical genomics 2008;2:48.

[7] Zhao C, Arroyo-Mora LE, DeCaprio AP, Sharma VK, Dionysiou DD, O'Shea KE. Reductive and oxidative degradation of iopamidol, iodinated x-ray contrast media, by fe(iii)-oxalate under uv and visible light treatment. Water Research 2014;67:144-153.

[8] Zhao C, Pelaez M, Dionysiou DD, Pillai, SC, Byrne JA, O'Shea KE. Uv and visible light activated tio2 photocatalysis of 6-hydroxymethyl uracil, a model compound for the potent cyanotoxin cylindrospermopsin. Catalysis Today 2014;224:70-76.

[9] Liu S, Zhao Y, Ma F, Ma L, O'Shea K, Zhao C, Hu X, Wu M. Control of microcystis aeruginosa growth and associated microcystin cyanotoxin remediation by electron beam irradiation (ebi). RSC Advances 2015;5(40):31292-31297.

[10] Zhao C, Sharma V, Dionysiou D, O'Shea K. In Oxidation of cylindrospermopsin and its model compound 6-hydroxymethyl uracil by ferrate (vi), Abstracts of Papers of the American Chemical Society 2014; American Chemical Society, Washington, DC, USA.