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Research Article

DRUGS AND CHROMATOGRAPHY: A CASE STUDY

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ABSTRACT

According to the United Nations world drug report 2013, "kratom" compounds, which consists of mitragynine and its major metabolite, 7-hydroxymitragynine a novel type of drugs which extracts from a tree planted in Southeast Asia area. It is necessary and urgent to develop novel methods which can be used for separation and detection of kratom compounds. The goal of the current project is to achieve such aims by utilizing high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS).Linearity was found between 10 – 500 ng/mL range and other analytical parameters were determined as well.

Keywords: Kratom, chromatography, mass spectrometry, case study.

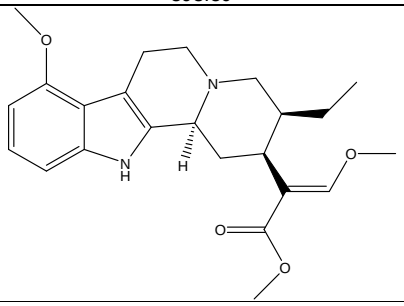
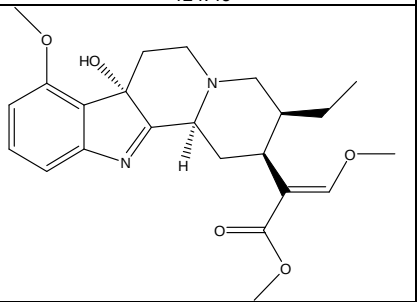
1. INTRODUCTION

Chromatography, including liquid chromatography, gas chromatography, supercritical fluid chromatography, and a number of other forms, is a separation technique which widely used in different areas of applications. Recent years, mass spectrometry becomes a popular detector coupling to the chromatographic systems.

According to the United Nations world drug report 2013, "kratom" compounds, which consist of mitragynine and its major metabolite, 7-hydroxymitragynine, are a novel type of drugs which extracts from a tree planted in Southeast Asian areas. It is necessary and urgent to develop novel methods which can be used for separation and detection of kratom compounds.

Researchers have reported a large number of scientific approaches for the determination of mitragynine and 7-hydroxymitragynine, including gas chromatography, liquid chromatography, capillary electrophoresis, and different techniques coupling to mass spectrometry and other technique methods [1-6].In this paper, the author describesa rapid approach to separate and identify the kratom compounds by new instrumentation-high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS).

Table 1: Information about the Kratom compounds

Items	Mitragynine	7-Hydroxymitragynine
Formula	$C_{23}H_{30}N_2O_4$	$C_{23}H_{30}N_2O_5$
Molar weight	398.50	414.49
Structure		

2. Experimental Section

The experiments were carried on an Agilent 1290 Infinity LC Systems (Shanghai, China) coupling to a Sciex API 4000triple quadrupolemass 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 was optimized to achieve rapid and effective goals for the objectives using direct infusion techniques before exploring UHPLC parameters. The mass spectrometer parameters are listed here.

The LC-MS was set under positive electro-spray ionization mode (ESI+) for both mitragynine and 7-hydroxymitragynine compounds. The electro-spray ionization is a soft ionization technique for mass spectrometry, which allows users to see the molecular ion peaks rather than the fragment peaks. The precursor ions, which were previously called as parent ions, were first explored to identify the compounds present in the samples. The precursor ions for mitragynine and 7-hydroxymitragynine are 399.1 and 415.1, respectively. The product ions, previously named as daughter ions, are 159.0 of mitragynine, and 190.3 of 7-hydroxymitragynine. The ionization voltage was set at + 3500 V for both mitragynine and 7-hydroxymitragynine compounds. The ionization temperature was set at 550 °C for both mitragynine and 7-hydroxymitragynine compounds.

Detailed mass spectrometry parameters were also examined. For instance, the sheath gas, which is a nitrogen make-up gas flow, was set to 30 for both mitragynine and 7-hydroxymitragynine compounds. Collision energy (CE), which is the most important tandem mass spectrometry parameter, was set to 60 for mitragynine, and 55 for 7-hydroxymitragynine.

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 developmentand stored at -4°C to keep the active component effective.

3. RESULTS AND DISCUSSION

Bothe mitragynine and 7-hydroxymitragynine were eluted with mobile phases of water with 0.01% formic acid as mobile phase A) and acetonitrile as mobile phase B). Fig. 1 demonstrates 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 5 min.A 3-minutepost run was employed after both compounds eluted.

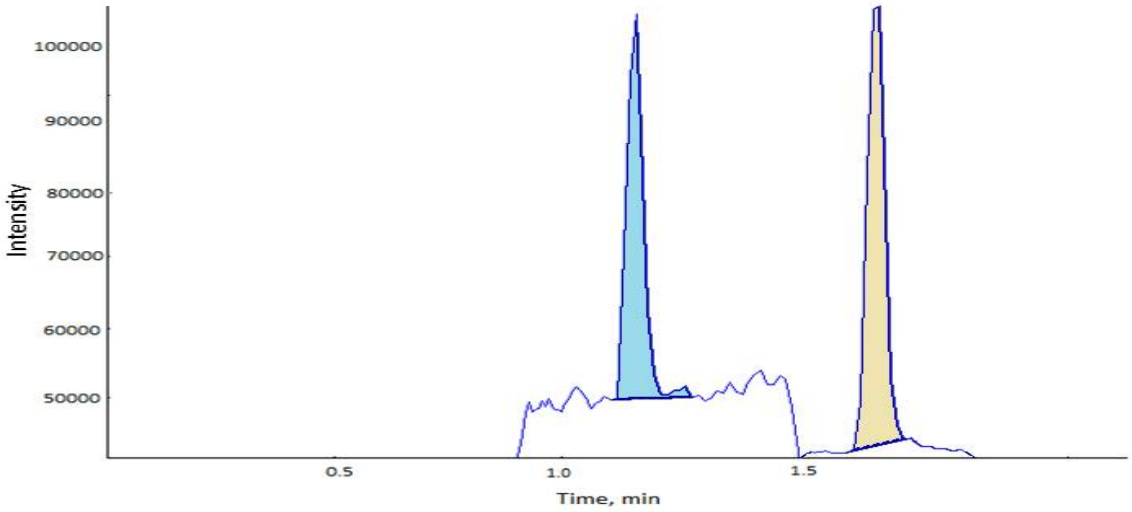


Fig. 1: Chromatogram of optimized conditions of separation and identification of mitragynine and 7-hydroxymitragynine compounds

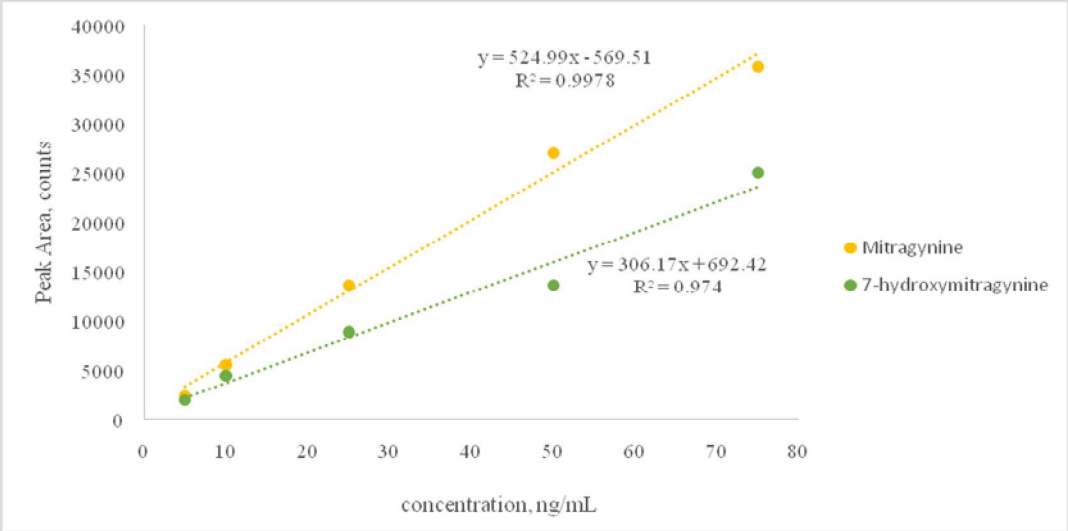


Fig. 2: Demonstration of linearity tests of mitragynine and 7-hydroxymitragynine compounds

4. CONCLUSIONS

This paper demonstrates an effective and rapid method for the separation and detection of kratom compounds including mitragynine and 7-hydroxymitragynineutilizing liquid chromatography-tandem mass spectrometry (LC-MS/MS). This optimized approach can be applied for various fields of applications.

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