Analysis of Mitragynine and Other Alkaloids in *Mitragyna speciosa* Plants Using Supercritical Fluid Chromatography

Application Note

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Abstract

The FDA has called for the detention of all imports of dietary supplements and bulk dietary ingredients that are or contain *Mitragyna speciosa* or the natural product kratom derived from this plant, due to the toxicity of kratom in multiple organ systems. The toxicity and psychoactive properties of this plant necessitate improved analytical techniques for the separation of the indole and oxindole compounds that are its major active components. A supercritical fluid chromatography (SFC) method using a diode array detector provided complete resolution of all eight compounds analyzed in this study, including three sets of diastereoisomers, in 7 minutes.

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Introduction

The Southeast Asian plant *Mitragyna speciosa* is of particular interest due to its psychoactive properties. It is the source of the natural product known as kratom or ketum, which is an easily obtained and low cost substitute for opioids. While multiple studies have shown that kratom is addictive [1,2], it is also used to alleviate the withdrawal symptoms of opium and decrease dependence on other drugs [3-5]. However, the US Food and Drug Administration (FDA) has determined that there is a lack of evidence to ensure that the use of kratom as a dietary ingredient is safe. The FDA has stated that the scientific literature identifies a number of health concerns, including respiratory depression, nervousness, agitation, aggression, sleeplessness, hallucinations, delusions, and a plethora of other afflictions associated with the consumption of kratom. As a result, the FDA has mandated the detention of all imports of dietary supplements and bulk dietary ingredients that are or contain *M. speciosa*, or the natural product kratom [6]. There is a potential for abuse due to the ready availability of this previously uncontrolled psychoactive plant.

The threat to human health posed by *M. speciosa* necessitates effective analytical methods for detection of its major active components. Although mitragynine and its derivative 7-hydroxymitragynine are the primary psychotropic constituents of kratom, other alkaloids present in *M. speciosa* include speciogynine and speciociliatine, which are diastereoisomers of mitragynine, as well the diastereoisomers paynantheine and 3-isopaynantheine. The indole alkaloids corynoxine and corynoxine B are also psychotropic components of kratom.

The analysis of mitragynine is commonly done using ultra-high performance liquid chromatography (UHPLC) methods. However, these methods have some disadvantages, including being relatively slow, requiring significant amounts of organic eluent, and lacking in specificity. This last drawback is due to the similarity of both the UV and mass spectra of the mitragynine diastereoisomers. Using gas chromatography (GC), the alkaloids of interest can be analyzed with high efficiency and without derivatization, making GC an attractive technique for these analyses. However, standard capillary columns may not provide adequate resolution of alkaloid diastereoisomers, such as mitragynine and speciociliatine. Due to the high temperatures needed to elute the alkaloids, GC method optimization is limited by the temperature range of the instrumentation.

Supercritical fluid chromatography (SFC) may be able to overcome the problems presented by HPLC or GC analysis of these indole alkaloids, particularly the resolution of diastereoisomers. SFC commonly uses CO_2 as a liquid eluent due to its low viscosity and high diffusivity, which enables the use of very small particle columns without extraordinary pumping pressures. The application of SFC often results in faster, more economical, and less environmentally harmful separations than those performed using HPLC, and the results are often orthogonal.

This application note describes the use of SFC for the separation of mitragynine, its diastereoisomers, and other indole and oxindole alkaloids in *M. speciosa* extracts, which has been published previously [7]. This SFC method provided complete resolution of all eight compounds of interest in 7 minutes, with no more than 15 % methanol in the eluent. SFC provided better resolution of the *M. speciosa* alkaloids, and was faster than the UHPLC and GC methods to which it was compared.

Experimental

Sample preparation

The leaves of *M. speciosa* were extracted using methanol as described [7].

Standards

Seven oxindole and indole alkaloids were isolated from the leaves of *M. speciosa* at the National Center for Natural Products Research (University of Mississippi) as described [7]. The alkaloid 7-hydroxymitragynine was purchased from Chromadex (Santa Ana, CA). A solution of all eight alkaloids (Table 1) was prepared and analyzed with the SFC, UHPLC, and GC methods, as described [7].

Table 1. Eight Indole and Oxindole Alkaloids Present in M. Speciosa

	Alkaloid	
1	Corynoxine B	Diastereoisomer sets
2	Corynoxine	
3	Paynantheine	
4	3-Isopaynantheine	
5	7-Hydroxymitragynine	
6	Mitragynine	
7	Speciogynine	
8	Speciociliatine	

Instruments

The SFC method was performed using an Agilent 1260 Series SFC/LC system employing an SFC pump with a backpressure regulator (previously Aurora), a quaternary LC pump, a binary LC pump for control of the CO_2 and modifier flow rates, and a diode array detector (DAD) as described [7]. The UHPLC and GC methods were also performed using Agilent instruments as described [7]. The SFC run conditions are shown in Table 2.

Table 2. SFC/DAD Run Conditions

Column	Agilent Rx-Sil column, 2.1 × 50 mm, 1.8 µm (p/n 827700-901)
Column temperature	25 °C
Backpressure	180 bar
Flow rate	0.5 mL/min
Injection volume	2 µL from a 5 µL fixed loop
Eluent	CO_2 with methanol containing 10 mM ammonium acetate
Gradient	6 to 15 % methanol in 10 minutes
DAD	10 mm 13 µL cell

Results and Discussion

SFC analysis of mitragynine in plant material

This was the first reported application of SFC for the analysis of mitragynine in plant material [7]. It was used to separate both a mixture of oxindole and indole alkaloid standards, and an alkaloid-enhanced extract of kratom. Using an Agilent Rx-Sil column and CO_2 with methanol containing 10 mM ammonium acetate as the eluent, mitragynine (6) was well-resolved from its diastereoisomers, speciogynine (7), and speciociliatine (8) (Figure 1A). The other five indole and oxindole alkaloids, including two sets of diastereoisomers, were also fully resolved. Separation was effected in a very short time (7 minutes), with minimal use of organic solvent (15 % methanol at the end of the gradient), and the elution pattern was orthogonal to that obtained with UHPLC.

This SFC/DAD method provided superior resolution to both UHPLC/DAD and GC/MS [7]. While the major alkaloids were well-resolved, the UHPLC method provided only partial separation of the mitragynine and speciogynine (6 and 7) stereoisomers, and no separation of the paynantheine and 3-isopaynantheine (3 and 4) stereoisomers (Figure 1B). The elution order was also very different from that obtained with the SFC/DAD method. The use of MS/MS could increase the selectivity and sensitivity of this approach, but would increase the cost and complexity of the analysis. A companion study [8] demonstrated that electrospray ionization (ESI) and collision induced dissociation mass spectrometry were not capable of differentiating any of the alkaloid diastereoisomers from each other, making identification very difficult using UHPLC/MS/MS.

The resolution of *M. speciosa* alkaloids is inadequate with GC as well (Figure 1C), as corynoxine B, and corynoxine (1 and 2), mitragynine, and speciociliatine (6 and 8) are only partially separated. Improvement of the separation by adjustment of parameters is severely hampered by the high temperature required to elute the alkaloids and the upper temperature limit of the polymeric GC stationary phase. In fact, in a gaseous system using a liquid stationary phase, resolving mitragynine and speciociliatine is difficult if not impossible [7]. Electron

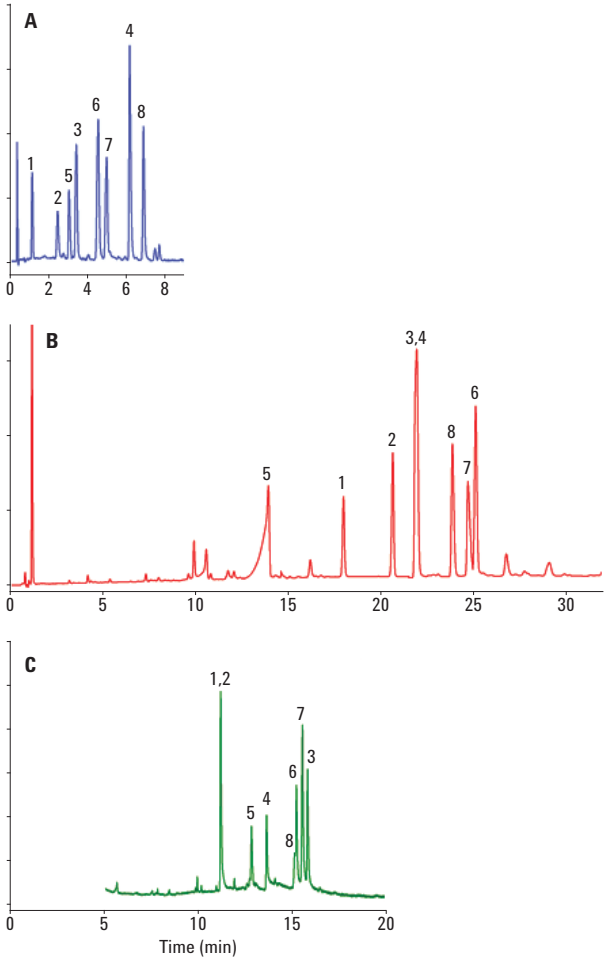

Figure1. Chromatograms of the M. Speciosa alkaloid standard mixture. A) SFC/DAD; B) HPLC/MS; C) GC/MS. The compound numbers are listed in Table 1. Note that the SFC run is done in the time required for just the solvent delay with GC/MS.

ionization (EI) mass spectra of alkaloids 6–8, which are available in many GC/MS libraries, are similar. While relative ion abundance can be used to differentiate mitragynine and speciociliatine, mass spectral analysis cannot enable quantification of these stereoisomers in the absence of complete chromatographic resolution [7].

Because of the complete resolution of the indole and oxindole alkaloids obtained by SFC analysis, it was possible to determine the relative distribution of all of the alkaloids within a given extract of *M. speciosa*. The distribution in a typical plant extract sample is shown in Figure 2.

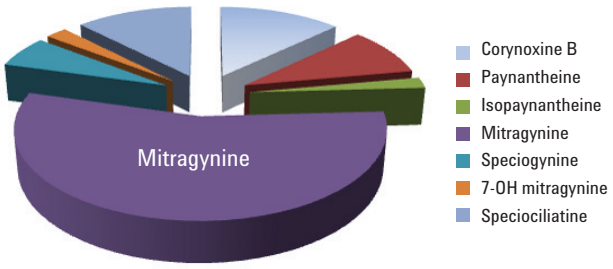

Figure 2. Typical distribution of alkaloids in an extract of M. speciosa as determined by SFC. Corynoxine was not detected in this particular sample.

Conclusions

While SFC has not been readily used for the analysis of kratom alkaloids in plants, it provides an effective means of resolving all eight compounds analyzed in this study, using liquid carbon dioxide modified with an organic liquid as the eluent. This approach is simple, using only a DAD, and fast, with separations being completed in approximately 7 minutes, and it uses less organic solvent than HPLC. Both conventional HPLC and the benefits of mass-specific detection can be used with this technique as well to assist in accurate identification and quantification.

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