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Note d'application

UPLC-MS/MS Analysis of Salvinorin A from *Salvia Divinorum*

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Abstract

This application note demonstrates the use of the ACQUITY UltraPerformance LC (UPLC) System coupled with tandem mass spectrometry to provide positive identification and of *Salvia divinorum* via the analysis of salvinorin A using a simple extraction technique. Amounts of salvinorin A in the plant material were also calculated using the same chromatographic run.

Benefits

- The extraction method was straightforward and the UPLC separation was rapid
- Methanol/water blanks showed no carryover, reducing the likelihood of false positives

Introduction

Salvia divinorum is a perennial herb native to certain small areas of southern Mexico. It is a large plant with large green leaves, and can be grown outside of its native habitat. *Salvia divinorum* is known to be a psychoactive herb and is used by the native Mazatec Indian Shamans to create altered states of consciousness during spiritual healing and other spiritual ceremonies and rituals. The active hallucinogenic component of *Salvia divinorum* has been identified as salvinorin A, Figure 1, (Siebert, 1994).

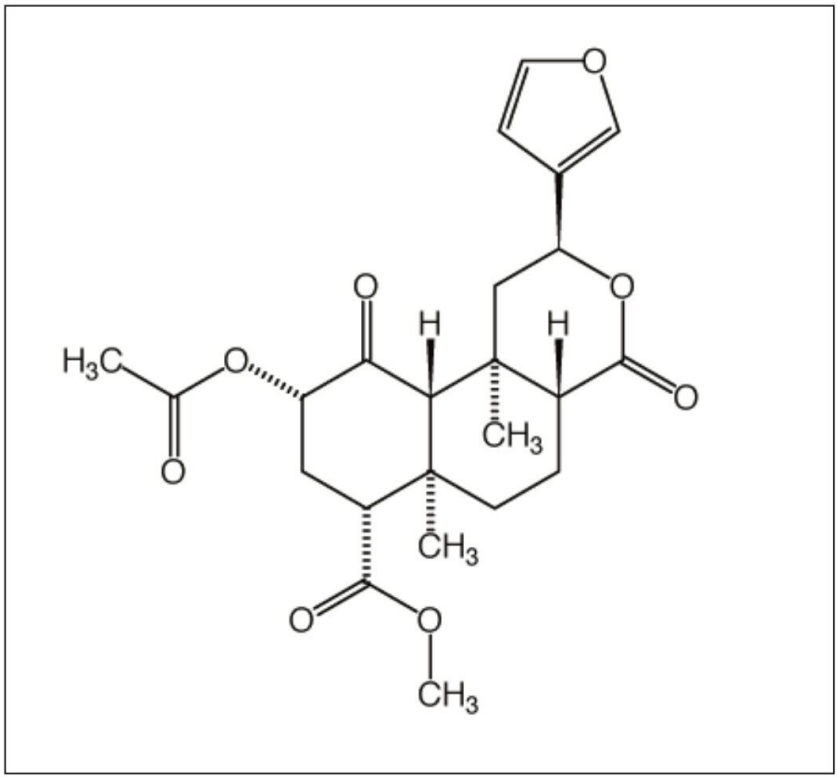

Figure 1. Chemical structure of Salvinorin A.

Currently, neither *Salvia divinorum* nor any of its constituents, including salvinorin A, are controlled under the federal Controlled Substances Act (CSA) within the United States, although the U.S. Drug Enforcement Agency (DEA) does consider *Salvia divinorum* to be a drug of concern. Several states (Louisiana, Missouri, Tennessee, Oklahoma, Delaware, Maine, and North Dakota) have laws prohibiting possession of *Salvia divinorum* and salvinorin A and other states are considering legislation regarding this plant. A number of countries (Australia, Denmark, Iceland, Finland, Norway, Sweden, Italy, Japan, Brazil, South Korea, United Kingdom, Estonia, Belgium, Spain) have laws that prevent the sale, distribution, importation, and possession of *Salvia divinorum* and salvinorin A.

When dried plant material becomes crushed or powdered many of the unique identifying anatomical features of the plant can become obscured.

This makes chemical identification of unique plant phytochemical constituents one of the few ways to confirm a plant's true identity. Because there may be legal implications for the possession of *Salvia divinorum* and salvinorin A, the confirmation of identity must be reliable and irrefutable.

Salvia divinorum can be identified by analyzing for the presence of the active component, salvinorin A, which

is unique to *Salvia divinorum*. Mass spectrometry, in combination with liquid chromatography (LC-MS) can provide positive, unambiguous confirmation of the presence of salvinorin A in the sample matrix, and just as importantly, provide evidence that plant material does not contain salvinorin A and therefore is not *Salvia divinorum*.

This application note demonstrates the use of the ACQUITY UltraPerformance LC (UPLC) System coupled with tandem mass spectrometry to provide positive identification and of *Salvia divinorum* via the analysis of salvinorin A using a simple extraction technique. Amounts of salvinorin A in the plant material were also calculated using the same chromatographic run.

Experimental

UPLC Conditions

LC system:	ACQUITY UPLC System
Column:	ACQUITY UPLC HSS T3 2.1 X 30 mm, 1.8 µm
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in acetonitrile
Flow rate:	0.60 mL/min
Gradient:	15% B to 90% B over 1.5 min
Injection volume:	1.0 µL
Sample temp.:	10 °C
Column temp.:	50 °C
Detection:	ACQUITY TQD

Data:	Empower 2 Software

MS Conditions

MS system:	ACQUITY TQD
Ionization mode:	ES+
Acquisition mode:	Multiple Reaction Monitoring MRM 1 433.4 > 373.2 MRM 2 433.4 > 323.1 MRM 3 433.4 > 295.2
Capillary voltage:	3.65 kV
Cone voltage:	10 V
Desolvation gas:	N_2, 550 L/hr
Cone gas:	N_2, 50 L/hr
Source temp.:	100 °C
Desolvation temp.:	550 °C
Collision gas:	Argon, 0.30 mL/min

Extraction

Dry *Salvia divinorum* (leaf and stems) samples and non-*Salvia divinorum* material were finely ground with a mortar and pestle. Portions (10 mg) were added to a 1.5 mL polypropylene snap cap centrifuge tube. To each tube was added 1 mL of methanol/water (9:1) and the tube and contents sonicated in a heated (35 °C) water bath for 1 hour. Following centrifugation, sample extracts were diluted 1/10 with extraction solvent and injected directly. Analyses were performed in triplicate.

Results and Discussion

Salvinorin A was separated from any interfering substances quickly using UPLC. Utilizing UltraPerformance Liquid Chromatography enables assays to be performed in a rapid manner without sacrificing separation quality. In this method, the UPLC run time was 90 seconds.

Three Multiple Reaction Monitoring (MRM) transitions were monitored from salvinorin A (Figure 2). The primary transition (433.4 > 373.2) was used for quantification and confirmation, while the other two transitions were only used for peak confirmation purposes. Peak height ratios of the primary and secondary MRM transition calculated using an authentic standard of salvinorin A were determined to be 6.1 ±0.8.

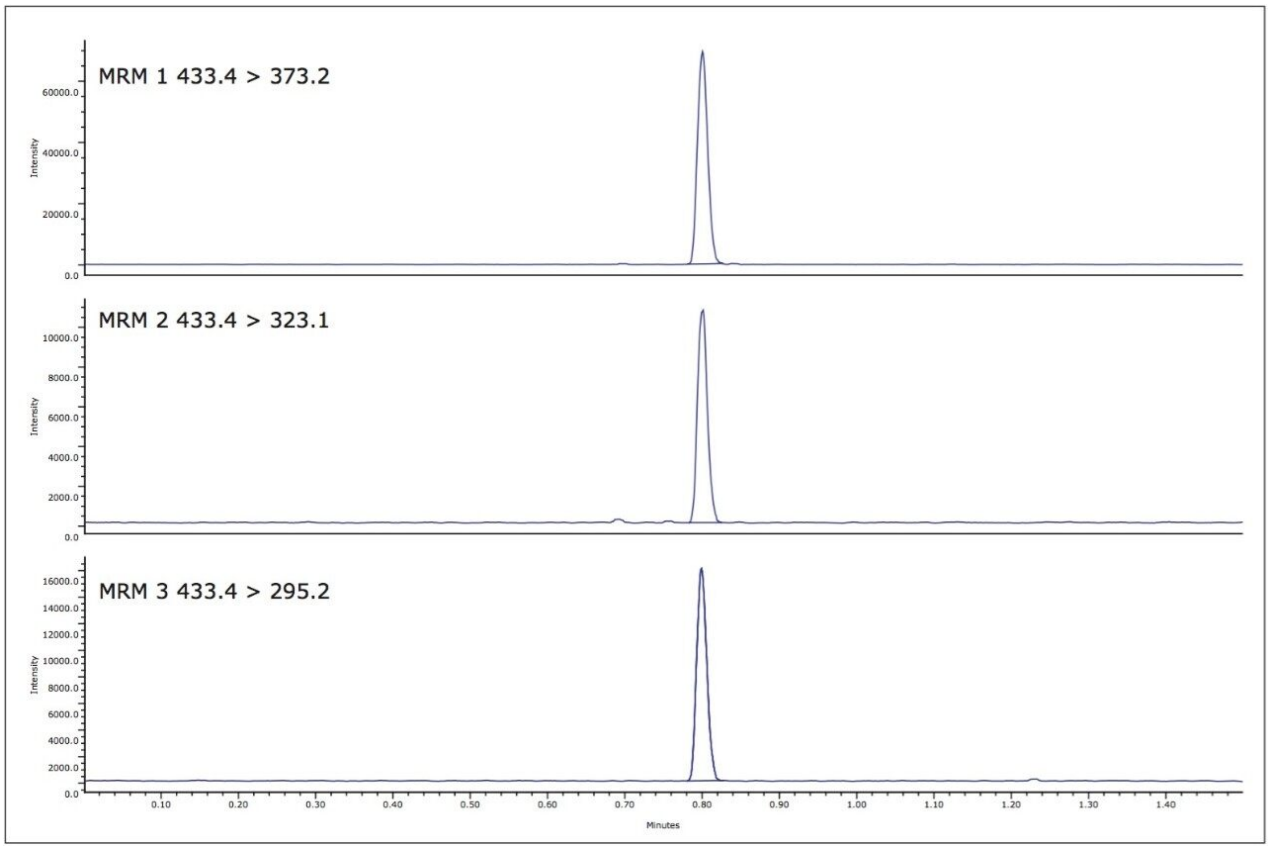

Figure 2. MRM chromatograms of Salvia divinorum extract.

The presence of salvinorin A in the ground plant material samples was considered to be confirmed by the following criteria:

1. All three monitored MRM ions were present.

2. A chromatographic peak was present at a retention time of 0.8007 ±0.0040 minutes (~0.5% RSD)

3. Peak height ratios of the primary and secondary MRM transition (373:323) were within ±20% of the calculated ratio which was determined to be 4.9–7.3.

A total of eight samples were analyzed. Three were dry, whole plant *Salvia divinorum* from different internet suppliers, two others were described by the suppliers as "enhanced" leaf (prepared by extracting salvinorin A, and adding it back into the leaves), and three were non-*Salvia divinorum* material (common sage, whole mint leaf, and ground mint).

Injections of sample extracts were bracketed by injections of authentic salvinorin A (Sigma-Aldrich Chemical Co.). Methanol/ water blanks were also injected throughout the sample set to assess carryover.

As can be seen from Table 1, salvinorin A was confirmed to be present in the five samples claimed to be *Salvia divinorum*. Ratios of the two MRM transitions fell between the prescribed levels, and retention times of the peak of interest were within the defined limits. Salvinorin A amounts were calculated from a five-point linear calibration curve (R^2 = 0.9975) based on the primary MRM transition (Figure 3).

Peak Height						
	Retention Time (min)	MRM 1	MRM 2	MRM 3	Ratio MRM1:MRM2	mg Salvinorin per Gram of Leaf
Sample Name n=3						
Sage Leaves	Not Found	0	0	0	Not Found	Not Found
Mint Leaves	Not Found	0	0	0	Not Found	Not Found
Mint Flakes	Not Found	0	0	0	Not Found	Not Found
S. divinorum 1	0.7999	23078	3609	5139	6.41	3.93 ±0.59
S. divinorum 2	0.7999	29788	4575	6172	6.43	5.59 ±0.36
S. divinorum 3	0.7991	20933	3278	4531	6.59	3.53 ±0.26
Enhanced Leaf 1	0.7994	65207	10106	13671	6.82	14.63 ±0.94
Enhanced Leaf 2	0.7999	97930	14553	21093	6.21	27.09 ±2.18

Table 1. Results of UPLC-MS/MS analysis of Salvia divinorum.

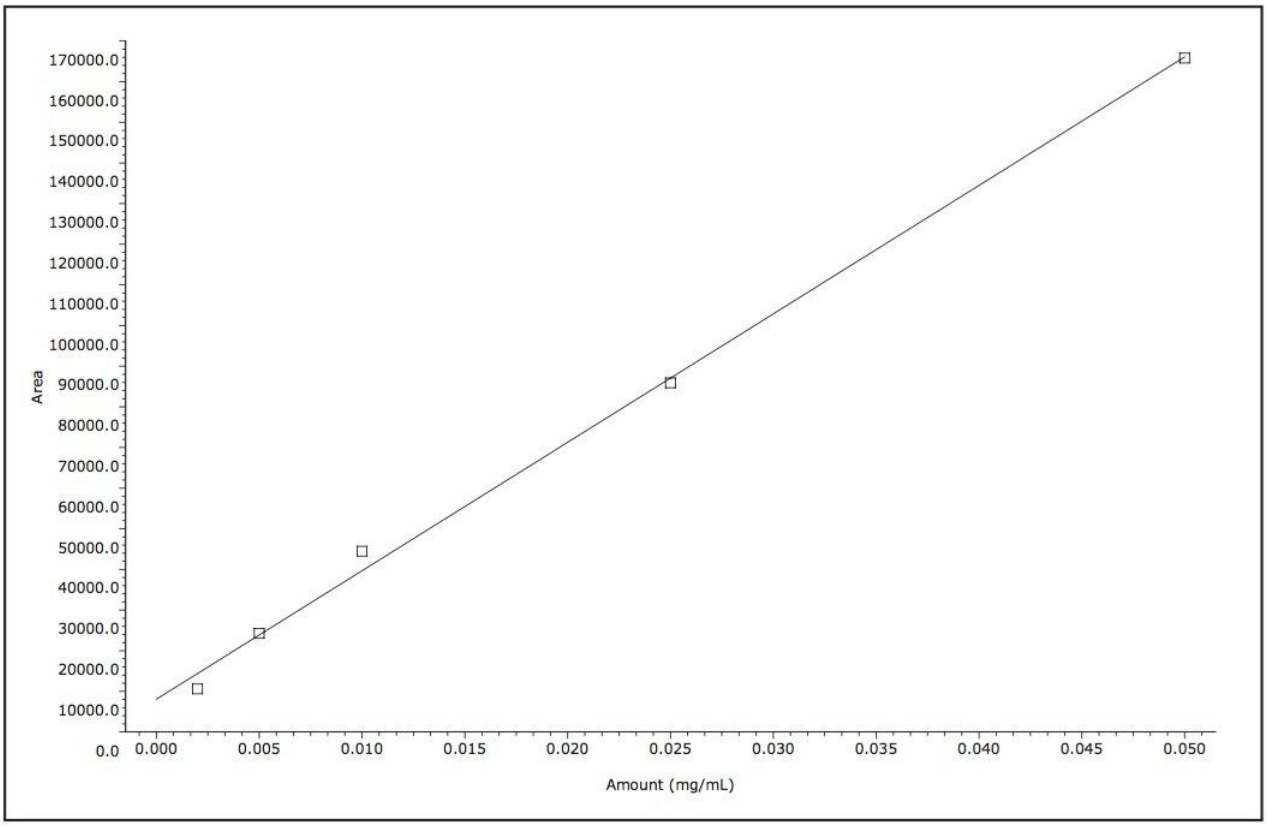

Figure 3. Salvinorin A calibration curve 433.4 > 373.2.

Standard concentrations ranged from 0.002 mg/mL to 0.050 mg/mL. Salvinorin A amounts in the leaf material ranged from 3.9 to 5.6 mg/g (dry weight) for unaltered material and 14.6 and 27.1 mg/g for the two enhanced leaf samples. Variability between different lots of plant material has been noted previously (Gruber *et al*, 1999). As expected, salvinorin A was not found in the common sage, mint leaf, and mint flake samples.

Using a weak wash solvent of 90:10 water/methanol and a strong wash solvent of 90:10 methanol/water; extraction solvent blanks run directly after the most concentrated standard and the final enhanced leaf extract showed no measurable carryover (Figure 4) proving the methodology has a low probability of false positives.

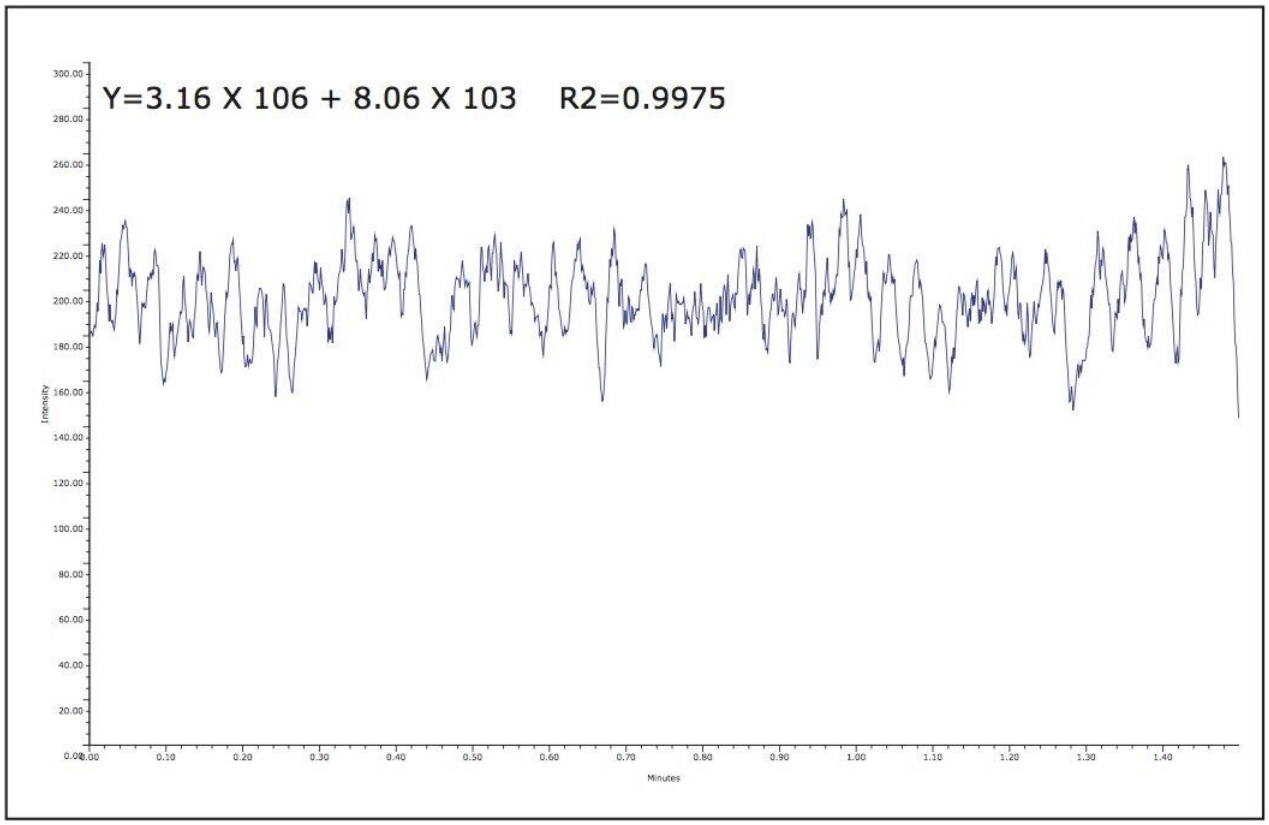

Figure 4. MRM1 chromatogram of methanol/water blank.

Conclusion

- Quantitation and confirmation of salvinorin A from *Salvia divinorum* using an ACQUITY TQD System was demonstrated in a single analysis
- The extraction method was straightforward and the UPLC separation was rapid (run time of 1.5 minutes)
- Unambiguous confirmation of the presence of salvinorin A was demonstrated using a combination of exceptional retention time reproducibility, three MRM transition ions present, and stable ion ratio between the primary and secondary MRM transitions
- Methanol/water blanks showed no carryover, reducing the likelihood of false positives

References

1. Siebert DJ. *Salvia divinorum* and salvinorin A: New Pharmacologic Findings. *Journal of Ethnopharmacology*. 1994 June; 43(1): 53–6.
2. Gruber JW, Siebert DJ, Der Marderosian AH, Hock RS. High Performance Liquid Chromatographic Quantification of Salvinorin A from Tissues of *Salvia divinorum*. Epling & Játiva-M. *Phytochem. Anal.* 1999; 10: 22–25.

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